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Manuscripts should be submitted to R. E. Buchanan, 24 Curtiss Hall, Iowa State College, Ames, Iowa.

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WINTER BEHAVIOR OF THE RING-NECKED PHEASANT, PHASIANUS COLCHICUS, AS RELATED TO WINTER COVER IN WINNEBAGO COUNTY, IOWA[1]

CARROLL R. GRONDAHL[2]

Iowa Cooperative Wildlife Research Unit
Department of Zoology and Entomology
Iowa State College

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Game managers generally acknowledge winter as a critical season in the life of the ring-necked pheasant, *Phasianus colchicus,* and they realize the importance of adequate cover in juxtaposition to a stable food supply, such as standing field corn, *Zea mays.* Also, it is recognized that long daily and/or weekly movements of pheasants on an area are, in part, an expression of the suitability of winter cover and food conditions (2, 7).

The purposes of this study were (*a*) to obtain information regarding the winter behavior of ring-necked pheasants in relation to farm shelterbelts and varying weather conditions, and (*b*) to contribute information for formulating management policies for this important game species. The investigations were conducted on 2,480 acres of the Winnebago Pheasant Research Area, Eden Township, Winnebago County, in north central Iowa from December 21, 1950, until June 1, 1951.

METHODS

Trapping operations were conducted at eight farm shelterbelts and one slough area with seven Ohio-type pheasant traps (6) during the period December 21, 1950, to March 3, 1951. The term *farm shelterbelt* as used in this paper denotes human plantings of trees adjacent to farm sites.

An Iowa State Conservation Commission aluminum butt-end leg band, a colored plastic leg band (10), and a slightly modified Taber marker (8) were attached to each trapped bird for identification purposes.

Early in the study a route was established that covered 21 miles of roads on and surrounding the area. The route was laid out in such a way that 17 miles were on roads circumscribing the area at distances

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[2] The author is indebted to Drs. E. L. Kozicky and G. O. Hendrickson under whose leadership the investigation was conducted. Their contributions of time in the field, counsel, and encouragement were of unlimited value. The kindness, hospitality, and material help extended by Mr. and Mrs. O. B. Christenson and the many other farmers residing on the study area are gratefully recognized.

up to 2.75 miles from the trap sites, while the remaining four miles were on roads traversing the area. The primary purpose for establishing the route was to make observations on the movements of marked birds. Similar information was recorded when the roads on the area were being traveled during the performance of other field duties. Also, farmers residing on the area cooperated by reporting observations of marked birds. A 6 $\times$ 30 binocular field glass was used to check birds for markers and bands and to observe pheasant behavior. The aluminum butt-end leg band was used as the basic criterion in determining whether a bird was marked.

Additional information on pheasant movements was obtained from marked birds retrapped, lost markers found in the field, and marked birds found dead on the area. Data from the last two sources were obtained while the investigator made a daily reconnaissance of the fields on foot during the winter and spring phases of the study. In the spring when the snow cover was disappearing, the entire area was systematically covered at least twice by the author in the search for dead birds and for markers lost from birds.

The winter population for the 2,480-acre area was derived by totaling the highest roosting counts taken at the farm shelterbelts and other concentration points during the month of January. These roosting counts were taken at daybreak and as shortly thereafter as possible.

The spring breeding population estimates per section were determined by recording the number and approximate location of all crowing cocks heard during a ten-minute period at each of five different stations. Four of these stations were located at the half-section lines and the fifth at the center of the section. While driving between the first four stations and walking to the fifth, the locations of all observed male birds were also recorded. On the basis of these data, the approximate number of males per square mile could be determined. The observed winter sex ratio was then applied to compute the total number of birds per section. A cock crowing call route (3) and a male roadside count (5) established in 1950, were run on ten mornings during the period May 5 to May 24. The information obtained was used as a check against the breeding population estimates. The observed winter sex ratio was determined by a combination of field and roadside observations (5).

WEATHER

Weather data were obtained from the U. S. Weather Station at Forest City, Iowa, approximately 20 miles southeast of the area. December, 1950, was cold and dry. Temperatures were 7.3 degrees (F.) below the 44-year normal. January, 1951, had a temperature 3.9 degrees below normal, and precipitation was 0.25 inches above normal. Snowfall totaled 12.8 inches and snow depth reached a maximum of 13.0 inches. February temperatures were 0.6 degrees above normal. Snow totaling 8.5 inches fell on six days. The latter part of the month was characterized with temperatures above normal. March had the greatest snowfall, 21.0 inches,

with seven days having .01 inches or more of snow, and some of the most severe storms of the winter. Temperatures were 10.7 degrees below normal. Spring thaws and periodic rains left the roads impassable during the first half of April. Temperatures were 5.6 degrees below normal and precipitation was 4.2 inches above normal. The total snowfall only amounted to 3.0 inches.

COVER OF AREA

There were 14 farm shelterbelts, one willow fence row, and two sloughs on the 2,480-acre area which were considered to be potential

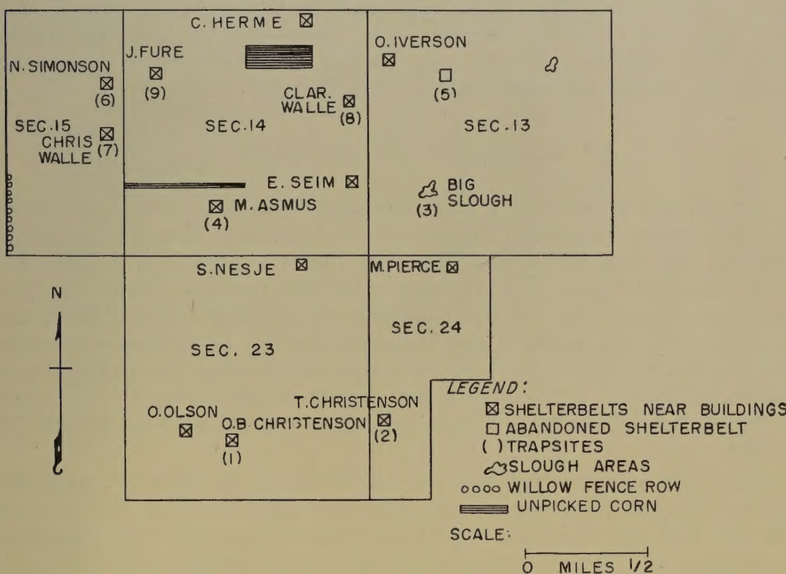

FIG. 1. Study area. The Winnebago Pheasant Research Area, Eden Township, Winnebago County, Iowa. December 21, 1950 — May 31, 1951.

winter concentration points for pheasants (Fig. 1). In general, the shelterbelts were planted about the time buildings were erected. Evidence of some natural reproduction, especially box elder, was apparent in many of the shelterbelts. Only limited replanting has been done. Species of trees most commonly found in the shelterbelts included: box elder (*Acer negundo*), American elm (*Ulmus americana*), Chinese elm (*Ulmus parvifolia*), green ash (*Fraxinus pennsylvanica* var. *lanceolata*), willow (*Salix* spp.), plum (*Prunus* spp.), and eastern cottonwood (*Populus deltoides*). Pine (*Pinus* spp.), spruce (*Picea* spp.), Catalpa (*Catalpa speciosa*), and maple (*Acer* spp.) occurred in lesser numbers.

The management of these shelterbelts is, for the most part, super-

ficial, with occasional cuttings being made to provide firewood. The shelterbelts are used for pasturing livestock with regularity on some farms. The areas of five shelterbelts ranged from 1.2 to 4.6 acres. One of the shelterbelts on the Otto Iverson farm is referred to as "abandoned" because it is not in the vicinity of farm buildings. The willow fence row, located in section 15, is a single row of trees 30 to 40 feet tall and about 440 yards long. The row extends from the south edge of section 15 north along the half-section line.

The two slough areas, both located in section 13, were 8.0 and 5.5 acres in area. These two areas are typical of the many sloughs that were once common throughout northwest Iowa and have since been drained. Several other small depressions are found on the study area but do not have sufficient vegetative growth to be considered as potential winter cover. The eight-acre slough area, located in the southwest quarter of section 13, will be referred to as the "Big Slough."

POPULATION AND SEX RATIO

A January census of the 2,480-acre area revealed a total of 508 birds; the 1950 winter population figure as determined by Kozicky and Hendrickson (5) was 435 birds. The 1951 figure represented an increase of 73 wintering birds (17.0 per cent) over that for 1950 (Table 1). The high percentage of birds found in section 14 was probably influenced by two unpicked corn fields, 2 and 15 acres. During 1951, counts revealed that 322 birds, 63.0 per cent of the entire population, were wintering in section 14; during 1950, this section contained 120 birds, 27.6 per cent of the winter population. There was no unpicked corn on section 14 in 1950.

The 1951 spring population was calculated to be about 60 birds per section. This figure represented an increase of approximately 16.0 per cent over the 1950 spring population figure and corresponded very closely to the increase of 17.0 per cent shown by the winter census.

The decrease in the number of birds from January to May of each year was approximately the same on a percentage basis. There was about a 55 per cent decrease in 1950 and a 54 per cent decrease in 1951. The winter mortality of 49 birds (9.65 per cent) in 1951 accounts for only part of this decrease. The two similar decreases suggest an ingress to the area in late fall or early winter and an egress in the spring. Possible reasons for a movement of this type are a readily available food supply or better winter cover conditions on the area. The first possibility was discounted because standing corn was available on the area in 1951 and not in 1950. The most plausible explanation was that the study area offered more suitable cover to wintering birds.

The winter sex ratio, based on roadside and field observations of 5,443 birds, was determined to be one male to 2.50 females, or 40 males per 100 females.

TRAPPING, BANDING, AND MARKING

A total of 230 birds was taken during trapping operations from December 21 to March 3. Traps were "set" a total of 199 days, giving a return of 1.16 birds per trap day. Of the total number of birds trapped, 197 (85.6 per cent) were new birds and 33 (14.3 per cent) were repeats. The new birds taken represented 38.7 per cent of the winter population of 508 birds. The sex composition of the newly trapped birds, 19 (9.6 per cent) males and 178 (90.4 per cent) females, showed a trapping selectivity or trapping site favoring females.

TABLE 1
Records of Maximum Observed Pheasant Populations on the 2,480-Acre Winnebago Pheasant Research Area

Cover Type	Section	Pheasant Population	
		Feb. 1950	Jan. 1951
Farm shelterbelt			
Asmus, M.	14	100	134
Christenson, O. B.	23	30	3
Christenson, T.	24	40	12
Fure, J.	14	0	48
Herme, C.	14	0	3
Iverson, O.	13	5	18
Nesje, S.	23	15	0
Olson, O.	23	25	4
Pierce, M.	24	60	26
Seim, E.	14	0	0
Simonson, N.	15	5	25
Walle, C.	14	20	137
Walle, C.	15	50	67
Abandoned farm shelterbelt			
Iverson, O.	13	25	2
Fence row (willow)			
Walle, C.	15	60	0
Slough			
Christenson, O. B.	13	.....	29
Total	.....	435	508

Of the 197 new birds trapped, 188 were banded with the Conservation Commission butt-end leg band and a colored plastic leg band. The difference of nine birds between those trapped and banded represented the number of new birds that were found dead in traps. Birds that were marked with Taber markers, in addition to the two types of leg bands, totaled 179. A shortage of the right color combinations of markers developed on several occasions where large catches were made. However, metal and plastic bands were attached to all birds regardless of whether markers were available or not.

WINTER MOVEMENTS

Pheasant movements on the area were determined from a total of 314 records of marked birds. These records were obtained from the following four sources: (1) roadside and field observations of marked birds, (2) marked birds retrapped, (3) lost markers found in the field, and (4) marked birds found dead on the area.

The mean daily cruising radius, based on 119 roadside and field observations of marked birds during the period December 21 to February 25, was 0.39 mile. One hundred and eight, 90.8 per cent, of these were observed within 0.75 mile of the place where marked. Weston (10) found that 139 birds traveled an observed mean distance of 0.46 mile from Grass Lake, Emmet County, Iowa, during the winter period of 1949–50.

Eight other records of marked birds at distances up to 2.0 miles were obtained during the winter period on the Winnebago Area. These were not used in figuring the mean daily cruising radius, for it was believed that they represented permanent winter movements. Four observations of birds at 1.75 to 1.99 miles were made of birds from the Big Slough in section 13, when it became unsuitable for roosting cover. Four other observations at distances of 1.00 to 1.24 miles were of birds from the M. Asmus shelterbelt in section 14. These four records were taken on different days at the southwest quarter of section 23, and it is possible that these records were of the same bird. Permanent movements of this type would erroneously increase the distance of the observed daily cruising radius.

The retrapping of 33 marked birds from December 21 to March 3 provided additional information on the movements of birds. Fourteen of the birds were taken at the same site at which they were marked, while 19 were taken in other traps. The greatest movement shown by these records was between the Big Slough and the abandoned shelterbelt in section 13 and the Clarence Walle shelterbelt in section 14 (Fig. 2). There were six instances of movement between the Clarence Walle and the abandoned shelterbelt and four cases of movement between the abandoned shelterbelt and the slough. In both cases the shortest distance between the two points was 0.40 mile.

Early morning counts indicated that the abandoned shelterbelt was used very little for roosting (Table 1). However, based on counts at different times of the day, this shelterbelt was used as a loafing site by birds. Some birds roosting in the Clarence Walle shelterbelt fed in the picked corn that bordered the abandoned shelterbelt on the west and south. Also, birds utilizing the slough area ranged north into the same picked cornfield. Trapping of marked birds at the abandoned shelterbelt showed that birds from the Big Slough and the Clarence Walle shelterbelt used this area for loafing. This was further substantiated by two markers found in the abandoned shelterbelt and the observation of one marked bird just to the east of this shelterbelt.

The four records of birds marked at the Big Slough and later recaptured at the Clarence Walle shelterbelt were made on January 14, February 14, and two on March 3. These and the shift of one bird marked on December 27 to the Chris Walle shelterbelt in section 15 before January 10, a movement of approximately 1.25 miles, undoubtedly represented part of the winter movement from the Big Slough.

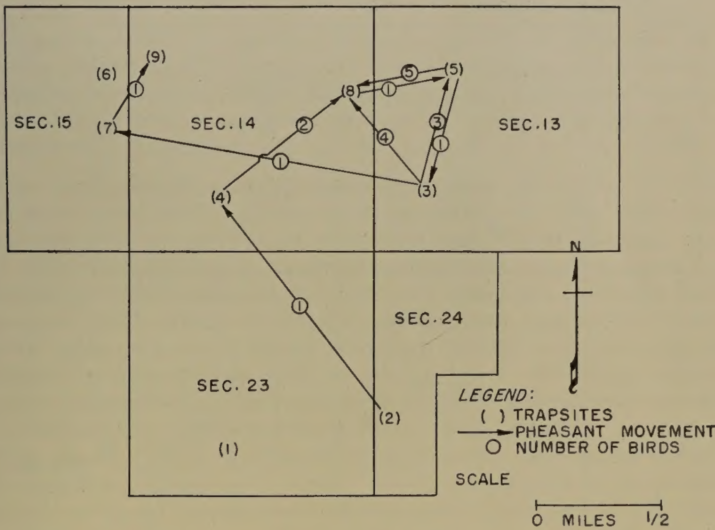

FIG. 2. Pheasant movement shown by the recapture of marked birds. The Winnebago Pheasant Research Area, December 21, 1950 — March 3, 1951.

Two records of birds moving from the M. Asmus shelterbelt to the Clarence Walle shelterbelt represented movement between the two shelterbelts harboring the two highest concentrations of birds and having a supply of standing corn close by. The distance between these shelterbelts is approximately 0.60 mile. One of the birds, a female, was marked on January 13 and recaptured two days later. The second bird, a male, was first taken on February 2 and recaptured on February 15, a lapse of 13 days. There is nothing to indicate that these movements represented anything but a normal shift of birds between concentration points.

The one record of movement from the T. Christenson shelterbelt to the M. Asmus shelterbelt in section 14 represented a movement of approximately one mile. This bird, a female, was first captured and marked on December 28 and recaptured on January 26. This movement followed a decrease in roosting activities at the T. Christenson shelterbelt as the winter progressed.

The one record of movement between the Chris Walle and the

J. Fure shelterbelts was of a female captured on February 2. The distance was approximately 0.40 mile. The length of time the bird had been banded was not determined, for the bird escaped before the band number could be recorded.

Included in the data obtained from the recapture of 33 marked birds were four individuals recaptured two times each and one that was recaptured three times (Fig. 3). All five of these birds were females. Of

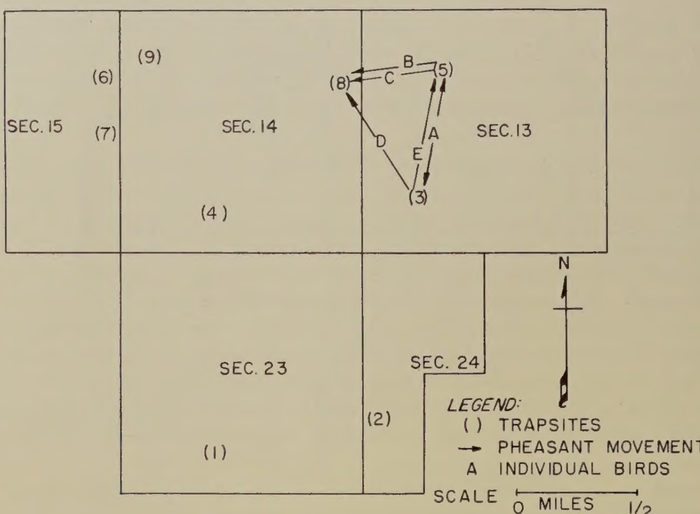

FIG. 3. Movements shown by pheasants recaptured two and three times. The Winnebago Pheasant Research Area, December 21, 1950 — March 3, 1951.

those retaken twice, one (A) was first caught and marked at the Big Slough on December 28. The following day it was recaptured at the abandoned shelterbelt. On January 2 it was retrapped again at the Big Slough, the original place of marking. Two of the other birds (B and C) were marked at the abandoned shelterbelt, and each was later recaptured two times at the Clarence Walle shelterbelt. Bird B was first taken on January 1 and then recaptured at the Clarence Walle shelterbelt on January 27, after an interval of 26 days. The second recapture was at this same shelterbelt on February 5. Bird C was marked on January 15 at the abandoned shelterbelt and was recaptured on February 4 and 14 at the Clarence Walle shelterbelt.

The fourth female recaptured two times (D) was marked on December 29 at the Big Slough in section 13. The first recapture was on January 4 at this same location, and the second was at the Clarence Walle shelterbelt on February 14.

The one bird that was recaptured three times (E) was marked on

December 29 at the Big Slough. The first recapture came three days later at this same location. It was trapped for the second time at the abandoned shelterbelt on January 11, and the third recapture was at the Clarence Walle shelterbelt on January 14. The records of these five birds further substantiate the idea that the abandoned shelterbelt was used as a loafing site by birds from the Big Slough and the Clarence Walle shelterbelt. They also indicate what Leopold, Lee, and Anderson (7) describe as a "circuit" type of movement. Regarding this, they say:

> Pheasants were sometimes restless in coverts of less than 10 acres. Where small coverts prevailed, both resident and released pheasants adoped a "circuit" type movement, traveling from one covert to another in a sequence spreading over a mile of distance and several days' time.

The most notable series of movements observed during the winter period were from the Big Slough area in section 13. The first roosting counts made on January 18 and 20 showed that 28 and 29 birds, respectively, were using this area. Previous to this time, a mid-morning count on December 25 revealed that 90 birds were using this cover for loafing. As the snow depth within the slough increased by snowfall and drifting, periodic visits revealed a steady decrease in the available vegetation which was suitable for roosting or loafing cover. Roosting counts on February 12 and 15 gave zero counts; inspection showed that very little of the original ground cover was available to the birds.

The observation of marked birds during the decrease in cover indicated a gradual dispersal of birds. The first indication of an abnormal movement (over 0.75 mile) came when a bird marked at the Big Slough was retrapped at the Chris Walle shelterbelt on January 10. This was a movement of approximately 1.50 miles. Following this, six other movements from 0.75 to 2.00 miles were observed. The variety of directions from the original place of marking in which the birds were observed indicated a random dispersal. The birds not only moved to the nearest points where roosting cover and food were available but to points beyond as well.

The absence of roosting birds at the Big Slough in February of 1950 (5) indicated a similar situation for that year. Slough vegetation of only eight acres evidently provides suitable loafing and roosting for pheasants in the fall and early in the winter; but as the season advances, drifting snow renders it worthless. This is at a time when cover is at a minimum, making it necessary for the birds to travel considerable distances in search of more adequate winter cover.

A winter movement of birds similar to that at the Big Slough was indicated for the T. Christenson shelterbelt in section 24. Counts during the first week of January showed that 12 birds were using these trees for roosting. On January 17 only three birds were recorded. The virtual abandonment of this shelterbelt was thought to be due to the decrease in the availability of food near the shelterbelt during the first part of

January as the snow depth increased to 13 inches. On January 26, a bird from this location was taken at the M. Asmus shelterbelt in section 14. This showed a northwesterly movement of approximately one mile. There was a two-acre field of unpicked corn adjacent to the M. Asmus shelterbelt. On January 19, a marked bird was observed at the west edge of section 24. This was a little less than one mile east of where the bird was originally marked. Leopold, Lee, and Anderson (7), in following the movements of released pheasants in Wisconsin, found that inadequate food induced prompt dispersal in seemingly random directions.

WINTER ROOSTING HABITS

A total of 66 roosting counts were made at 13 shelterbelts (Fig. 1) during the period of December 26, 1950, to May 17, 1951. These counts showed considerable variation. The highest were obtained during January and February in the majority of the shelterbelts (Table 2). The

TABLE 2

High Monthly Roosting Counts at Various Concentration Points in the Winnebago Pheasant Research Area, December 21, 1950—May 31, 1951

Roosting Area	Month					
	Dec.	Jan.	Feb.	Mar.	Apr.	May
Clar. Walle	....	137	103	52	18	5
M. Asmus	42	134	115	58	21	....
J. Fure	....	48	44	24	19	1
C. Herme	....	3	1	....	....	....
Chris Walle	....	67	48	24	17	....
N. Simonson	14	25	17	....	....	....
O. B. Christenson	....	3	0	19	5	....
O. Olson	....	4	....	....	....	....
S. Nesje	....	0	....	53	0	....
O. Iverson	....	18	21	25	....	....
Abandoned shelterbelt	....	2	....	....	....	....
T. Christenson	....	12	....	....	....	....
M. Pierce	11	26	33	37	3	....
Big Slough	....	29	0	....	....	....
Willow fencerow	2	....	9	....	....	....
Herme unpicked corn	....	....	0	0	0	....

table shows that considerably higher counts were obtained at the O. B. Christenson and S. Nesje shelterbelts during March. The increase in the first instance resulted when a number of birds moved in from a drainage ditch to roost in the trees. An additional ten inches of snow, with considerable drifting, was recorded during the week preceding the higher count.

The great increase of birds using the S. Nesje shelterbelt was thought to be part of the concentration that had been using the M. Asmus shelterbelt in section 14 earlier in the winter. This increase of roosting birds was of short duration and seemed to coincide with the beginning of the

winter flock break-up. The Asmus and Nesje farms are about 0.25 mile apart.

The birds representing the slight increase in roosting at the M. Pierce shelterbelt came from the Big Slough in section 13. The available cover in this area filled up with drifting snow about mid-February, causing the birds to move to other concentration points.

The Clarence Walle, J. Fure, and the M. Asmus shelterbelts in section 14, and the Chris Walle shelterbelt in section 15 were visited most frequently in making roosting counts. They were being utilized by the greatest numbers of birds. Temperature, wind velocity, precipitation, snow depth, and drifting snow were factors determining the numbers of birds using the wooded areas. Generally, roosting in shelterbelts was more prevalent when temperatures were below 20 degrees (F.), when winds were above 10 miles per hour, and when snow cover was greater than six inches (Table 3). The birds were found roosting in stubble, weedy fencerows, slough areas, and picked corn fields when the woody areas were not being used.

The portion(s) of any one shelterbelt used for roosting by the pheasants remained fairly constant throughout the winter. One exception was noted in the Asmus shelterbelt in section 14. A shift from the southeast to the northwest corner by the majority of the roosting birds was noted on several occasions when strong winds were blowing from the southeast. Females tended to concentrate in larger groups while some of the males very often roosted singly or in small groups apart from the females.

During the spring period, five of the shelterbelts were mapped and analyzed for possible factors responsible for their use by wintering birds (Table 4). Box elder trees, 24 to 30 years old and 25 to 30 feet tall, were the species most utilized for roosting in three of the shelterbelts, while plum trees, 12 to 18 years old and approximately 18 feet tall, were preferred in one. Generally, both of these species had similar growth habits in that they provided generous amounts of branching within 20 feet of the ground level.

The high winter count of pheasants for the C. Herme shelterbelt in section 14 was three birds. The limited use of this shelterbelt by pheasants was thought to be due to a combination of factors relating to the size of the shelterbelt, the proximity of farm buildings, the age and size of trees, and the species of trees present. Some definite values for these factors were derived by comparing the C. Herme shelterbelt with four others that were used by concentrations of pheasants ranging from 48 to 137. The larger of the two fields of unpicked corn (15 acres) — these fields were considered to be the primary reasons for the high concentration of pheasants in section 14 — was located approximately 400 feet to the south of the C. Herme shelterbelt. For this reason the availability of food was discounted as a factor responsible for the few birds using this wooded area.

The C. Herme shelterbelt was the smallest, 1.23 acres. The original planting was made in 1912, but at the time of study, an estimated 30 per

TABLE 3

Roosting Counts Taken at Three Shelterbelts in the Winnebago Pheasant Research Area, December 21, 1950—May 31, 1951

Date	Clar. Walle	M. Asmus	Chris Walle	Weather				
				Preced. Eve.				
				Wind (mph)	Temp. (F.)	Snow-fall	Drift-ing	Snow Depth (in.)
Dec. 27.	......	42	......	13 SW	4	......	x	5.0
Jan. 4.	......	55	......	8 N	10	......	x	9.5
9.	137	......	......	14 SE	8	......	x	13.0
11.	......	134	......	0	11	......	......	13.0
12.	......	......	14	5 S	18	......	......	13.0
22.	......	......	67	5 S	0	......	......	10.0
Feb. 2.	......	115	23	0	−12	......	......	11.0
3.	103	......	......	2 SW	6	......	......	10.0
5.	......	62	28	3 NW	12	......	......	10.0
6.	74	......	......	27 SE	20	......	x	10.0
9.	......	85	2	0	− 4	x	......	10.0
12.	93	......	......	8 NW	30	......	......	9.0
13.	......	......	48	24 NW	4	......	......	9.0
14.	......	0	......	7 NW	− 2	......	......	9.0
16.	......	4	2	0	32	......	......	9.0
20.	......	5	......	0	30	......	......	8.0
21.	0	......	......	10 NW	28	......	......	8.0
23.	......	10	4	3 S	35	......	......	7.0
24.	0	......	......	8 SE	34	......	......	6.0
26.	......	7	......	0	34	......	......	5.0
27.	4	......	......	7 NW	35	......	......	5.0
28.	......	0	16	0	38	......	......	5.5
March 2.	25	......	......	4 NW	20	......	......	7.0
5.	......	15	24	12 S	32	......	......	7.0
6.	52	......	......	19 SE	32	......	......	7.0
7.	......	32	18	10 NW	12	......	......	7.0
8.	34	......	......	7 N	18	......	......	7.0
9.	......	0	1	6 NW	8	......	......	7.0
10.	7	......	......	11 E	12	......	......	7.0
16.	......	48	15	0	32	......	......	12.0
17.	7	58	......	8 SE	28	......	......	12.0
28.	......	4	6	4 SE	38	......	......	17.0
29.	36	......	......	15 NW	32	x	x	17.0
30.	......	7	18	17 NW	32	......	......	15.0
31.	28	0	......	11 NW	28	x	......	15.0
April 2.	10	......	......	7 NW	32	......	......	......
3.	18	0	......	12 NW	34	......	......	......
4.	0	0	......	3 NW	34	......	......	......
9.	......	12	17	12 NW	32	......	......	......
10.	0	......	......	0	40	......	......	......
11.	......	3	2	10 NW	34	......	......	......
13.	16	21	......	24 NW	32	x	......	......
14.	......	0	1	5 NW	34	......	......	......
18.	......	0	2	12 SE	44	......	......	......
19.	4	......	......	4 NW	38	......	......	......
20.	......	0	0	0	40	......	......	......
23.	......	......	2	10 NW	36	......	......	......
25.	......	8	5	12 NE	38	......	......	......
27.	6	......	......	13 SE	50	......	......	......
28.	......	4	4	9 SW	68	......	......	......
May 12.	5	......	......	8 NW	54	......	......	......
17.	2	......	......	0	68	......	......	......

cent of the total area was made up of a ten-year-old planting of Chinese elm, American elm, and green ash. These trees ranged in height from 15 to 20 feet and were not considered as being valuable as roosting cover for wintering birds.

The next largest shelterbelt, located on the Clarence Walle farm, was 0.47 acres (38.2 per cent) larger than the C. Herme shelterbelt and harbored as many as 137 roosting birds during the winter. It is doubtful that the relatively small difference in size between these two shelterbelts could be a very important factor in limiting the use of the C. Herme shelterbelt to the extent shown by the roosting counts. However, the difference in size in combination with the planting of ten-year-old trees was thought to be important in explaining the limited use made of this shelterbelt by roosting birds.

Bue (1, p. 11) in studying the winter behavior of 600 to 1,000 birds concentrated on an area in Sully County, South Dakota, found very little use being made of young trees for roosting. Two shelterbelts, both planted in 1941, were located on the area. They included species of plum, box elder, Chinese elm, cottonwood, green ash, and Russian olive (*Elaeagnus angustifolia*) from 5 to 12 feet in height. Concerning these, Bue says,

> Only once during several night observations were birds roosting [in the trees], and then only 8 to 10 birds which represented a very small fraction of the concentration. Nearly all the birds roosted in [a] stubble field. . . .

Further evidence that young stands of trees do not provide pheasants with desired roosting cover was obtained on the Winnebago Area. Several observations at the E. Seim shelterbelt, also located in section 14, showed that pheasants were not using this area for roosting. The shelterbelt, which borders the farm buildings on the north and west sides, was planted in 1940–42.

At the C. Herme shelterbelt, the most distant trees from either house or barn were 252 feet. The same measurement taken at the other four shelterbelts varied between 306 and 450 feet. This indicated the close proximity of the C. Herme shelterbelt to the farm buildings. It seems logical to assume that the activity around the buildings on the Herme farm would be more disturbing to the pheasants.

Plum and box elder trees, which were the species of trees preferred for roosting at the other four shelterbelts, were found at the C. Herme shelterbelt. The plum trees, 8 to 18 feet tall, were located in an area 70 to 145 feet from the house, which was the closest of the buildings. The box elder trees, 10 to 25 feet tall, were located in an area 30 to 113 feet from the house. A few trees of this species less than 15 feet tall were interspersed with willows at distances up to 225 feet but were considered to be too small for roosting. At the farm shelterbelts in which roosting was common, the preferred trees utilized by pheasants for roosting were

TABLE 4

SHELTER DATA FOR THE WINNEBAGO PHEASANT RESEARCH AREA, WINTER, 1950–51

Shelterbelt	Area of Trees (*acres*)	Area of Farm Site (*acres*)	Most Distant Trees From House or Barn (*feet*)	Major Species Used for Roosting	Age of Major Species (*years*)	Height of Major Species (*feet*)	Minor Species Used for Roosting	Age of Minor Species (*years*)	Height of Minor Species (*feet*)	High Winter Count
M. Asmus Sec. 14	4.64	8.48	450	Box elder	25–30	30	Box elder	30–40	30	134
J. Fure Sec. 14	2.74	8.08	369	Box elder	24–30	25	Plum	15–25	25	48
Chris Walle Sec. 15	2.88	6.87	306	Box elder	25–30	25–30	Box elder	27–40	25–30	67
Clarence Walle Sec. 14	1.70	4.24	352	Plum	12–18	18	Box elder	17–30	18	137
C. Herme Sec. 14	1.23	4.46	252	....	....	....	....	....	....	3

located at distances of 108 to 345 feet from the closest building. On the basis of the information obtained from the shelterbelts that attracted roosting birds during the winter, the above figures indicate the potential roosting sites at the C. Herme shelterbelt were too close to the farm buildings to be utilized by pheasants.

Roosting in spruce trees was observed at three shelterbelts on the area. At the M. Pierce farm, roosting concentrations of as many as 37 birds regularly utilized a row of spruce on the west edge of the shelterbelt. The spruce trees were approximately 30 feet tall and the birds would concentrate about three-fourths of the way to the top. There were approximately 24 conifers in the shelterbelt, but only the spruce were being used for roosting. During January, at the T. Christenson shelterbelt, section 24, as many as 12 birds were flushed on several occasions from spruce trees at the north edge of the grove. Birds utilized about the same portion of the trees as they did at the M. Pierce shelterbelt. Several evening and morning observations at the O. B. Christenson shelterbelt in section 23 revealed three male birds using spruce trees directly in front of the house.

The last roosting counts, on May 12 and 17, showed that a few pheasants continued to roost in the shelterbelts up to the time that nests were being established. Two birds flushed from the Clarence Walle shelterbelt at daybreak on May 17 were roosting in box elder trees. The box elder trees started leafing out on May 3, indicating that a considerable amount of foliage was present on the trees when the last roosting counts were made.

SPRING MOVEMENTS

The mean distance of spring dispersal as shown by the observations of 87 marked birds from February 26 to May 31 was 0.55 mile. An observation on March 5 at a distance of 1.65 miles from the place of marking was the first indication of spring movement. The longest record of spring dispersal was 1.85 miles. Weston (10) found the spring dispersal began during March 6 to 12 at Grass and Birge lakes in 1949 and 1950. He separated the mean distances of dispersal into three groups: (*a*) records gathered between March 6 and 31, the period during which dispersal was just underway, (*b*) data for the April 1–30 period, gathered during the peak of dispersal, and (*c*) data for the remainder of the period each spring. These means were 0.55, 0.98, and 1.40 miles, respectively. The means for similar periods in the present study were 0.57, 0.58, and 0.52 mile, respectively. Aside from minor exceptions, Weston found that pheasants dispersed no more than 4.5 to 5.0 miles out on the farm land surrounding Birge and Grass lakes each spring.

The longer distance of spring dispersal observed by Weston may be attributed in part to the size of the two areas being used by the pheasants for winter cover. Grass Lake had an area of 173 acres; Birge Lake, 137 acres. These larger areas harbor larger concentrations of pheasants, and therefore, they attract birds from greater distances. Kimball (3) found

a maximum movement, based on crowing intensity samples, of ten miles from winter to summer range in South Dakota.

On the Winnebago Area, the observations of six birds at distances of 1.00 to 1.24 miles and four at 1.75 to 1.99 miles during the period December 21 to February 25 indicated that the maximum observed midwinter movement of birds can be expected to approach or even exceed the distance of mean observed spring dispersal. Long winter movements by pheasants are probably related to a search for more adequate food and cover as the winter progresses.

The numbers of birds observed during the two-week periods varied considerably. Between March 12 and April 22, continued snowfall and drifting followed by an extended period of thawing and 7.59 inches of rain resulted in many impassible roads; consequently, the daily roadside observations were not possible. Very few marked birds were observed during foot reconnaissance. Further, the number of marked birds observed during the spring period was limited by the number of markers that had been lost from birds by this time, and the difficulty in spotting the colored plastic leg band increased due to the greater amount of vegetation.

Certainly the data do not suggest the degree of movement shown by the spring census. These showed that approximately 54 per cent of the wintering birds were not present on the 2,480-acre area in the spring. A more distinct spring dispersal probably would have been revealed if the investigator had been able to observe a greater number of birds during the spring.

The recovery of 46 markers that had been lost from birds and 14 marked birds found dead in the field gave further evidence of pheasant movement. The records from these two sources did not show when movement occurred, for there was no way to determine how long the markers had been in the field before they were found. The rapid melting of snow resulted in an increase in the number of markers found after April 1. The maximum movement shown by these 60 observations was 1.49 miles, with 48 (80 per cent) of them under 0.75 mile.

Figure 4 shows graphically the direction and approximate distance the marked birds were recovered from their winter concentration points. Included are seven records received after the investigator left the area on June 1. All of these birds were females that were killed by mowers during the haying season. The other two recoveries after June 1 represented male birds killed during the hunting season. The longest movement recorded was one of the hunting kills. This bird was marked at the Clarence Walle shelterbelt and shot approximately two miles southeast of there nine months later.

In addition to the preceding observations, two unconfirmed reports of marked birds were received from individuals residing on the area. One was reportedly seen 4.5 miles northwest, and the other 2.0 miles north of the original marking sites.

A summary of all observations showed that 86 per cent of all records

of marked birds were taken within 0.75 mile of the place where marked.

SUMMARY

1. The purpose of this study on 2,480 acres of the Winnebago Pheasant Research Area, Eden Township, Winnebago County, Iowa, was (*a*) to obtain information regarding the winter behavior of ring-necked pheasants in relation to farm shelterbelts and varying weather conditions and (*b*) to contribute information for formulating management policies for this important game species.

2. There were 14 farm shelterbelts, one willow fencerow, and two

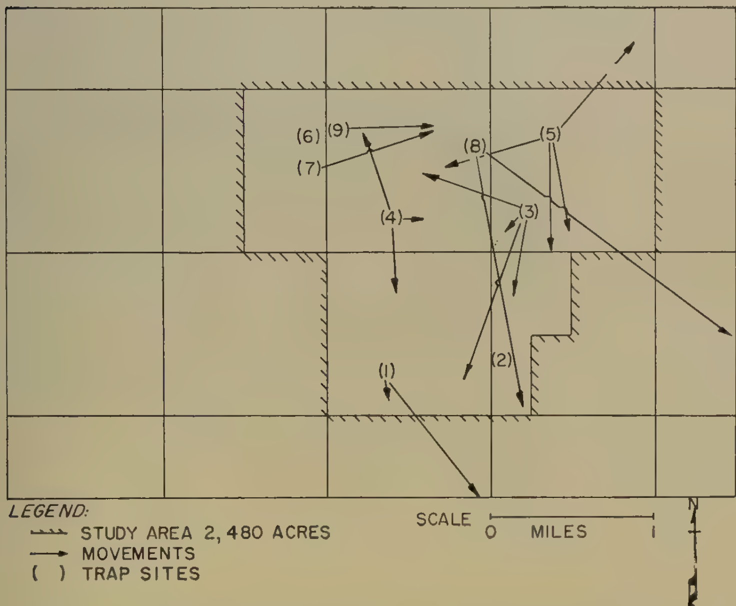

FIG. 4. Movements of pheasants shown by marked birds. The Winnebago Pheasant Research Area, December 21, 1950 — June 1, 1951.

sloughs on the area which were considered to be potential winter concentration points for pheasants.

3. Trapping operations were carried on at nine trap sites with seven Ohio pheasant traps during the period December 21, 1950, to March 3, 1951.

4. A total of 230 birds were taken during trapping operations. Of these, 197 (85.6 per cent) were new birds and 33 (14.3 per cent) were repeats. The new birds taken represented 38.7 per cent of the winter population.

5. A January census of the 2,480-acre area revealed a total of 508

birds on the area, or one bird per 4.88 acres. This represented an increase of 17.0 per cent over that for 1950.

6. The spring population was calculated to be 232.5 pheasants for the 2,480-acre area. This represented an increase of 16.0 per cent over the population for the same period in 1950.

7. There was a winter-to-spring decrease of 55 and 54 per cent for the years 1950 and 1951, respectively. This suggested a late fall or early winter ingress and a spring egress from the area. The most plausible explanation for this movement was more suitable winter cover on the area.

8. The mean daily cruising radius, based on 119 observations of marked birds during the period December 21 to February 25, was 0.39 mile. One hundred and eight of these were observed within 0.75 mile of the place where marked.

9. The retrapping of 19 birds at sites other than where marked indicated considerable movement between two shelterbelts and a slough and a shelterbelt. In both cases the shortest distance between the two points was 0.40 mile.

10. A winter dispersal of birds from a slough area was noted in January when this area filled up with drifting snow. The varied direction of movements up to 2.00 miles indicated a random dispersal.

11. Highest roosting counts were obtained during January and February for the majority of the shelterbelts. There was a general decline in shelterbelt usage in each succeeding month following January.

12. Generally, roosting in shelterbelts was more prevalent when temperatures were below 20 degrees (F.), when winds were above 10 miles per hour, and when the snow depth was greater than six inches.

13. Box elder trees, 24 to 30 years old and 25 to 30 feet tall, were the species most utilized for roosting in three shelterbelts, while plum trees, 12 to 18 years old and approximately 18 feet tall, were preferred in one.

14. The relatively small number of birds using the C. Herme shelterbelt was thought to be due to a combination of factors relating to the size of the shelterbelt, the proximity of farm buildings, and the species, age, and size of trees.

15. Spruce trees in three shelterbelts were used for roosting by 3 to 37 pheasants.

16. The mean distance of spring dispersal, as shown by the observations of 87 marked birds from February 26 to May 31 was 0.55 mile. The first indication of spring movement was on March 5.

17. The recovery of 46 markers that had been lost from birds and 14 marked birds found dead in the field showed a maximum movement of 1.49 miles. Forty-eight (80 per cent) of these were under 0.75 mile.

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POTENTIOMETRIC IODINE TITRATION OF BRANCHED STARCH FRACTIONS[1]

JOSEPH F. FOSTER AND RICHARD L. SMITH[2]

Department of Chemistry
Iowa State College

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The formation of the blue iodine complex by the unbranched portion of starch, amylose, is well known. A method for studying this complex formation in aqueous solution by potentiometric titration has been proposed by Bates, French, and Rundle (1) and is widely used. The branched fraction, amylopectin, gives only a reddish-violet coloration and binds little iodine under the conditions used by Bates *et al.*

In the case of amylose, the affinity for iodine appears to be very much dependent on chain length, and a possible mechanism has been proposed to explain this fact (1, 3, 6). The relatively weak affinity of amylopectin for iodine is in accord with its branched structure, but some binding might be expected by the outer free chain ends at high iodine levels. In the hope that a study of this binding might throw some light on the structure of amylopectin, attempts were made to extend the titration procedure to such materials.

In the concluding stages of this work a quantitative study of the binding of iodine by amylopectin and glycogen was reported by Higginbotham (5). His technique possesses certain advantages, particularly in the better control of iodide concentration. The method presented herein possesses the advantage of simplicity and speed and, at least at high iodide levels, gives results in substantial agreement with those of Higginbotham.

EXPERIMENTAL

Titration Arrangement. Attempts were first made to extend the titration range using a calomel reference electrode and titrating with iodine solution about ten times as concentrated as used in the Bates-French-Rundle technique. Precision at high iodine levels was poor. The method finally adopted was a modification of the differential titration method which has been used by Gilbert and Marriott (4) at very low iodine activities. Two 500-ml. round bottom three-neck flasks equipped with water sealed air stirrers (to minimize loss of iodine by volatilization) and connected by an agar-agar salt bridge containing saturated KCl were employed. It was found important to thermostat the cells because of

[1] Journal Paper No. J–1924 of the Iowa Agricultural Experiment Station, Ames, Iowa; Project 817. Supported in part by a grant from the Corn Industries Research Foundation. Taken from a thesis submitted by R. L. Smith in partial fulfillment of the requirements for the degree Master of Science, Iowa State College, 1950.

[2] Present address, Cook Paint and Varnish Co., Kansas City 10, Mo.

the large temperature coefficient of the binding reaction. (The temperature used was 25° controlled to about ± 0.03°.) The electrodes were of platinum foil one cm. square. The measuring circuit consisted of a Leeds and Northrup Type K potentiometer and a sensitive Leeds and Northrup galvanometer.

Procedure. The carbohydrate sample (40–500 mg.) was placed in 5.0 ml. of 1.0 N. KOH and allowed to stand until dissolved (usually several hours). It was then neutralized with 0.5 N. HCl to *p*H 6.3 (mixed methyl red indicator), sufficient 0.5 N. KI added to give the desired final normality, and diluted to 100 ml. The final solution was thus 0.05 N. with respect to KCl and 0.05–0.30 N. with respect to KI. A second 100 ml. of solution was prepared in precisely the same manner, except that carbohydrate was omitted and was added to the reference cell. The cells were assembled, flushed out thoroughly with nitrogen and maintained under a slight positive nitrogen pressure during the titration.

The titrating solution consisted of approximately 0.016 N. iodine containing KCl and KI of the same normality as that in the solution being titrated. In some cases iodine ten times as dilute was used in the early stages of the titration. Usually a total of approximately 100 ml. of iodine solution was added so that the final volume was doubled. Increments of iodine were added to the cell containing the sample and the E.M.F. brought to zero by titration into the reference electrode. Two to three minutes were allowed for equilibration before the final adjustment. It was assumed that at the null point both the iodine and iodide activities were the same in the two cells. (As will be seen below, this is probably not true in all cases.) On the basis of this assumption the free iodine activity in the sample cell was calculated from the amount of iodine added to the reference electrode and the total volume in the reference electrode. Iodine bound was calculated by subtracting free iodine from the total iodine added to the sample cell.

Samples used. Potato amylose and amylopectin samples were prepared from potato starch using the Pentasol fractionation procedure of Schoch (7). The other starch fractions were supplied by Dr. Schoch and were prepared by his earlier butanol procedure. The glycogen was a commercial preparation.[3] The waxy maize was a sample grown at Iowa State College and known to be relatively free of contamination by common corn starch.

The limit dextrins were prepared by digesting with crystalline β-amylase. The alcohol precipitable material was recovered, redissolved, and redigested a second time in the case of tapioca, twice more in the other cases.

RESULTS AND DISCUSSION

Effect of Iodide Concentration. The first titrations were carried out at 0.05 N. KI concentration. Up to iodine levels of almost 10^{-3} N., the resultant curves (of mg. iodine bound per 100 mg. sample versus moles

[3] Pfanstiehl Chemical Co.

free iodine per liter) were reasonable and appeared to be approaching a saturation value. However, when attempts were made to go to somewhat higher iodine concentrations, the curves were irregular and usually showed a tendency to drop. This could best be explained on the basis that iodide ions were also being bound.[4] A study was made at higher iodide levels using 100-mg. samples, and some of these results are reproduced in Figure 1. At 0.1, 0.2, and 0.3 N. KI the curves do not level off but continue to rise, as has been found by Higginbotham (5). The difference between 0.1 and 0.3 N. KI appeared to be so slight that 0.1 N. was used in most subsequent work. In retrospect, it appears that perhaps this concentration is not always adequate and that 0.2 N. KI should have been used.

Effect of sample size. Iodine binding curves are given in Figure 2 as determined from experiments on 40-, 100-, and 500-mg. samples all run in 0.10 N. KI. The effect of iodide binding is seen to be serious for the smallest sample, but the two larger samples are almost indistinguishable. Samples of approximately 100 mg. were used in most of the experiments, due to the difficulty of dissolving and working with the larger samples. In some cases there may be some loss of accuracy on this account but the preparation of the more concentrated amylopectin solutions posed a considerable problem.

Comparison of various starches and starch fractions. Figures 3, 4, and 5 give results on potato, corn, and tapioca starches, their amylose and amylopectin fractions, and the limit dextrins prepared from the amylopectins. In all cases the amount of iodine bound by the amylopectins is seen to increase almost linearly with the logarithm of the free iodine concentration, binding beginning usually in the neighborhood of 10^{-5} molar. The whole starches show two distinct titration regions with a fairly distinct break between the titration of amylose and amylopectin.

There are significant differences in binding affinity between the various amylopectins, potato binding at the lowest iodine activity, and corn at the highest. This was observed also by Higginbotham (5). A number of potato amylopectin subfractions (results not shown) prepared by fractional precipitation with ethanol appeared to have identical iodine binding curves except for differences in the degree of contamination with amylose.

It was hoped that some light might be thrown on the mechanism of binding by comparison of the amylopectins and the limit dextrins prepared from them. Thus, if binding is primarily due to outer (free) branches, it might be expected that it would be practically eliminated on removal of these branches by β-amylase. Higginbotham (5) has concluded on the basis of spectral absorption studies that two distinct modes of binding are operative in amylopectin, namely, helical binding of tri-iodide ions and adsorption of either tri-iodide ions or free iodine. The

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[4] It should be mentioned, though, that it appears necessary to assume the binding of much more iodide than was found by Higginbotham (5) to explain these results quantitatively.

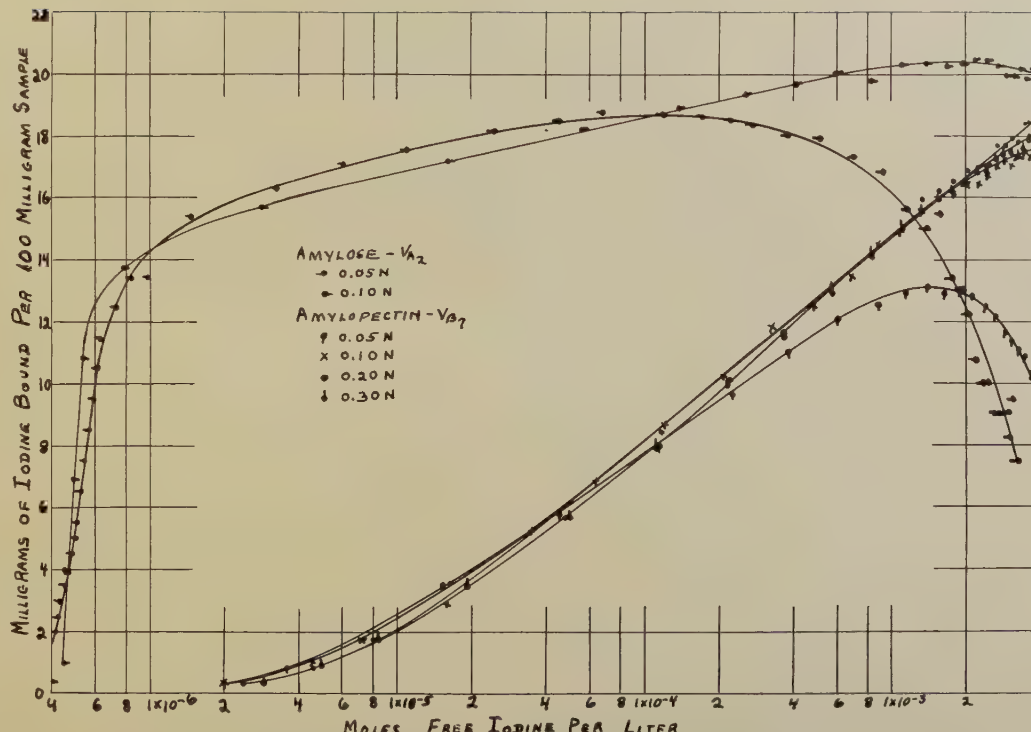

Fig. 1. Effect of iodide concentration on apparent iodine bound by potato amylopectin and amylose.

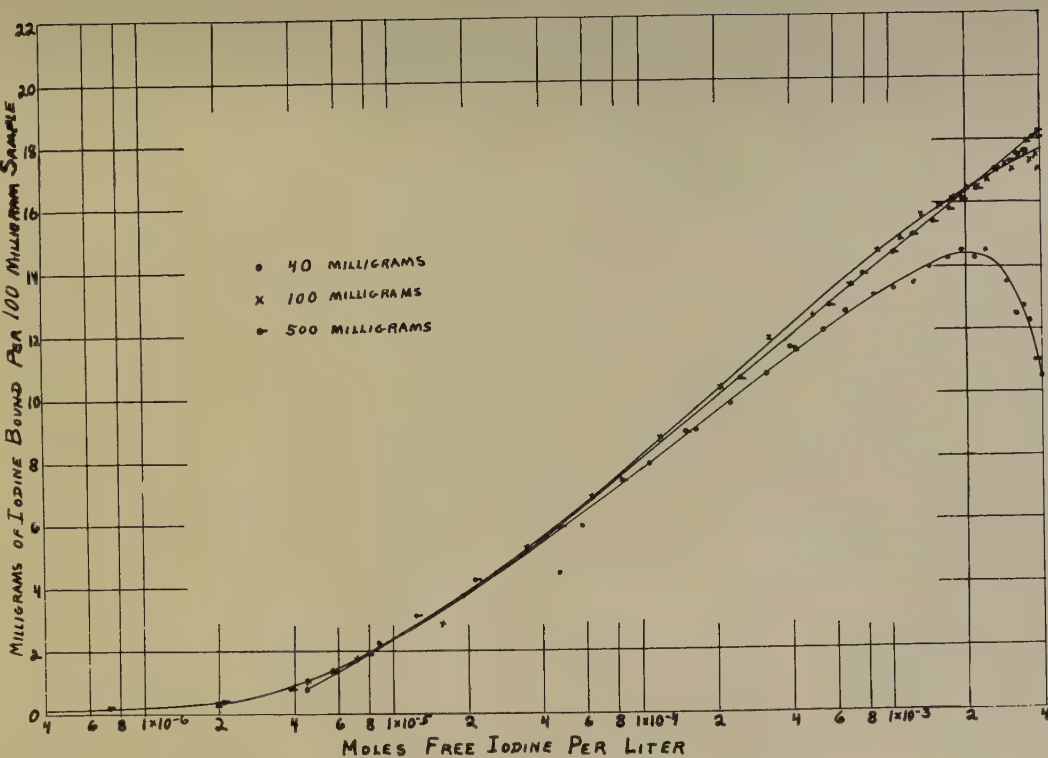

Fig. 2. Effect of sample size on apparent iodine bound by potato amylopectin.

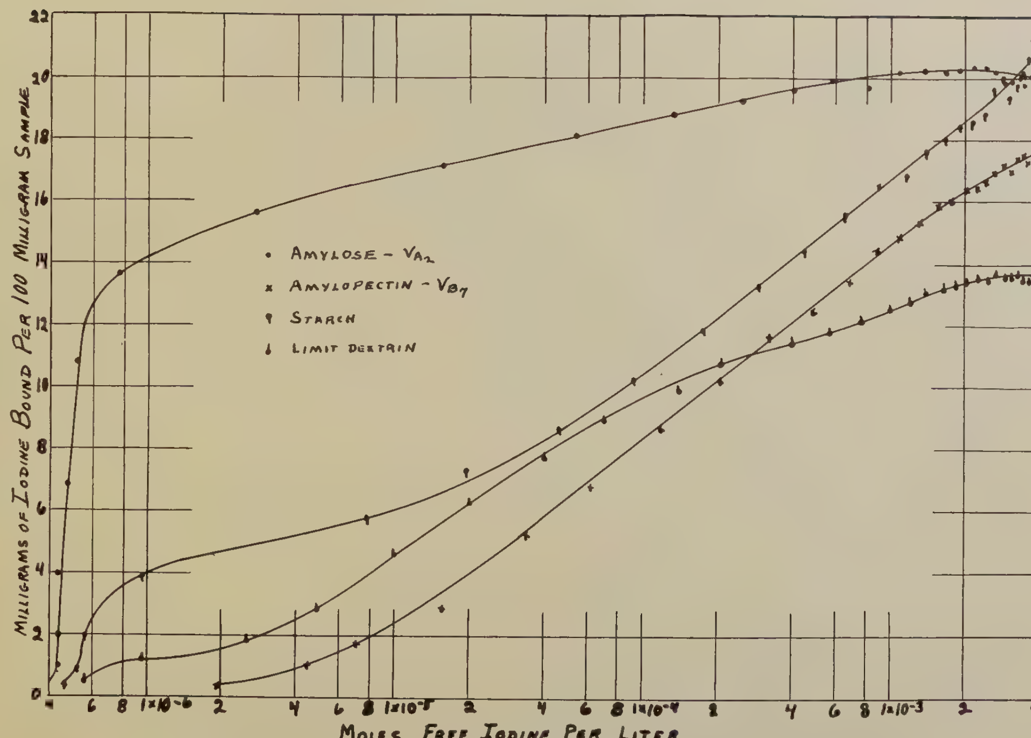

FIG. 3. Iodine binding by potato starch, its amylose and amylopectin fractions, and limit dextrin.

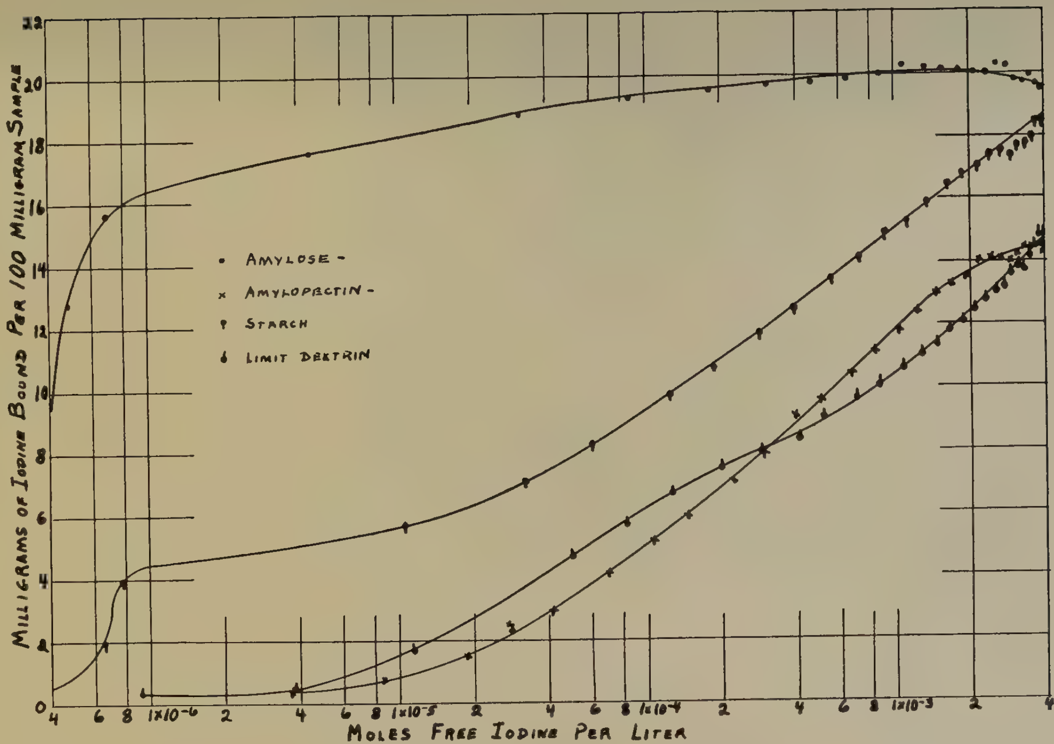

Fig. 4. Iodine binding by corn starch, its amylose and amylopectin fractions, and limit dextrin.

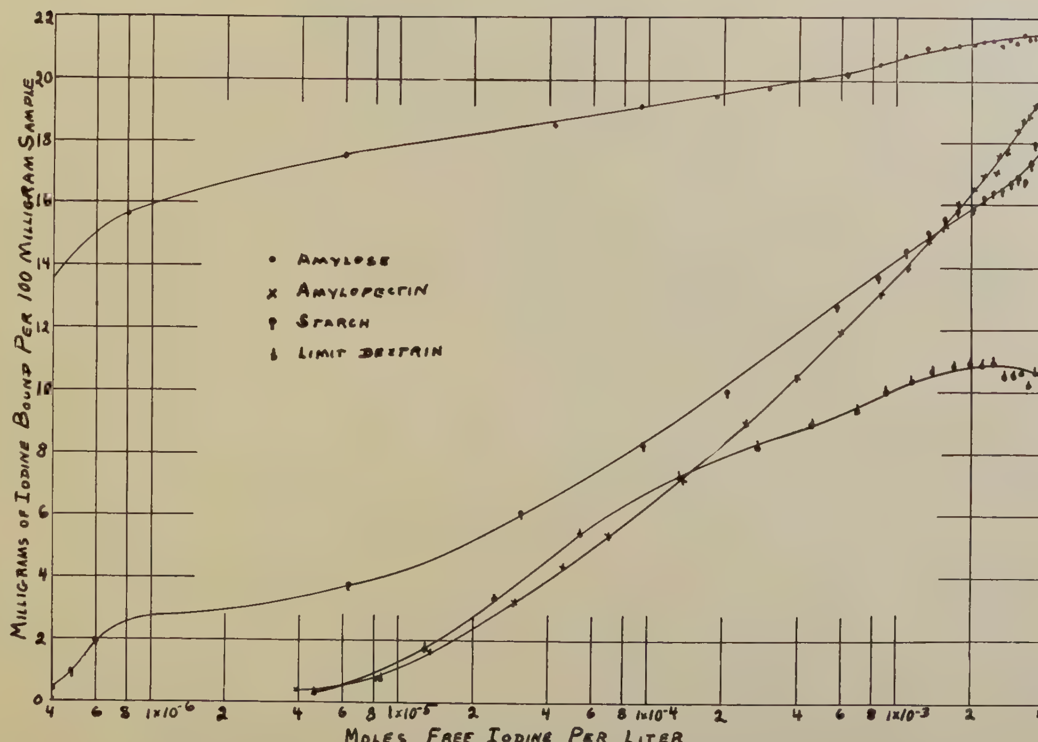

Fig. 5. Iodine binding by tapioca starch, its amylose and amylopectin fractions, and limit dextrin.

two mechanisms were thought to operate throughout the titration region studied, the proportion of iodine bound by the adsorption mechanism increasing with increasing total binding.

Actually, examination of Figures 3, 4, and 5 shows clearly that the binding in the first half or so of the titration is not reduced on removal of the outer branches; if anything, it is increased. The increase in the case of potato can probably be accounted for on the basis of an increased concentration of amylose in the limit dextrin,[5] which can be clearly seen in the titration curve. In the case of corn, the increased binding in this region appears to be significant, and in the case of tapioca, it may or may not be. On the other hand, in these three cases there is a pronounced decrease in binding ability at high iodine levels. These results do not appear to be in accord with the conclusions of Higginbotham (5) as to binding mechanism. However, it should be pointed out that digestion of the outer branches would expose other branches. These, to be sure, would have short side stubs but might nevertheless be capable of helix formation. These branches might be just as long as, or even longer than, the original external branches.

In the case of waxy maize, the titration curves of the limit dextrin and the parent starch are probably identical within experimental error (Fig. 6). Glycogen picks up iodine at even a higher iodine level than either amylopectin or the limit dextrins.

An unbranched amylodextrin prepared by acid degradation of granular potato starch and having a number average degree of polymerization of 14.5 glucose units was found to pick up iodine in the same region as the glycogen sample. It is of interest that this (14.5 glucose units) is very nearly the average number of glucose units per branch in glycogen, but the similarity in titration behavior may be quite fortuitous.

ACKNOWLEDGMENTS

The authors are indebted to Dr. T. J. Schoch of the Corn Products Refining Company who supplied most of the starch fractions studied. Dr. A. K. Balls of the Western Regional Research Laboratory kindly supplied the crystalline β-amylase preparation. The limit dextrins were prepared by Mr. Gene Wild of this laboratory. The critical comments of Drs. Dexter French and Eugene Paschall are appreciated.

SUMMARY

A differential null-point titration procedure for the study of iodine binding by amylose and amylopectin at relatively high iodine levels (above 0.001 molar) is described. The method, although suitable for routine measurements, yields results on various amylopectins which are in substantial agreement with those reported by Higginbotham using a more

[5] It appears that the amylose in the amylopectin is almost quantitatively present in the limit dextrin, presumably due to aggregation preventing its degradation by the enzyme (2).

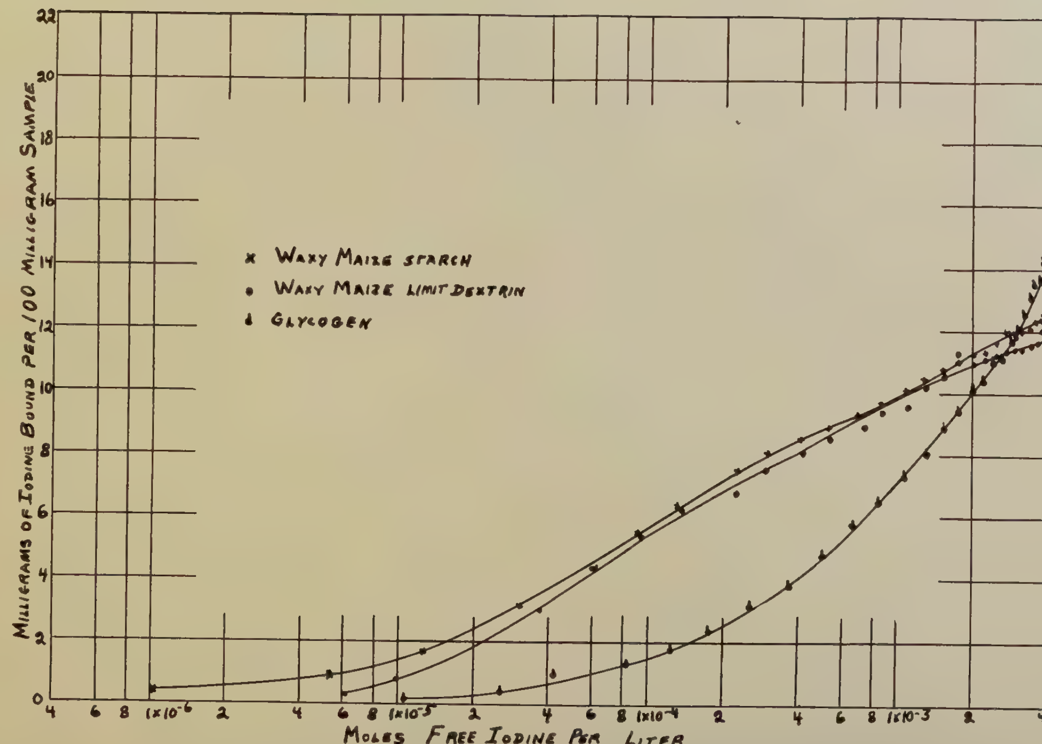

Fig. 6. Iodine binding by waxy maize starch and limit dextrin, and by glycogen.

precise but much more cumbersome method. Typical results are presented for the whole starches from potato, corn, and tapioca and for the corresponding amylose and amylopectin fractions and the limit dextrins. Results are also presented on waxy maize starch and its limit dextrin, and on glycogen. Binding by the limit dextrins is at least as strong, in the early stages of binding, as in the case of the parent amylopectins. The possible significance of this result in terms of the binding mechanism is discussed.

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METHODOLOGY FOR ISOLATING SALMONELLA FROM DRIED EGG PRODUCTS

John C. Ayres

Department of Bacteriology
Iowa State College

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Much evidence has accumulated during the past decade which strongly suggests that dried egg products serve as a vehicle for the dissemination of bacteria belonging to the genus *Salmonella* and that these products are responsible for certain outbreaks of food infection. Although cases of gastroenteritis caused by these organisms seem either to be increasing in number and significance or knowledge of their role in food products is being more generally recognized, the danger still is of unknown dimension. Hinshaw and McNeil (21) consider that the importance and incidence of *Salmonella* infections have been underestimated. Also, they are of the opinion that there is too great a tendency in the United States to characterize salmonellosis solely as a gastroenteritis inasmuch as Bornstein (7) clinically classified fever and septicemia as two other manifestations of the disease.

According to Edwards, Bruner, and Moran (12), birds constitute the greatest reservoir of potential *Salmonella* infections. Hinshaw and McNeil (20) reported that, of 58 types found in avian species, 50 were isolated from turkeys, 43 from chickens, and 13 from ducks. Fifty-two types of *Salmonella* were isolated from spray-dried whole egg powder by Solowey, *et al.* (39). In 1947, the Medical Research Council of Great Britain (31) and the Swedish Medical Board (40) implicated American dried egg products with outbreaks of food infections in those countries. Also, several workers (23, 32, 36, 40) have presented evidence which relates egg products to specific cases of gastroenteritis.

Experimental data of Haines and Elliot (19), Gibbons, Moore, and Fulton (17) and Solowey and Calesnick (38) show that *Salmonellae* multiply rapidly in reconstituted egg held at room temperatures. Solowey and Calesnick warned that rehydrated egg powder held for periods longer than four hours constitutes a critical potential hazard. The increased commercial production of dried egg products in the last twelve years further serves to emphasize this danger.

Inasmuch as *Salmonella* is a genus of nonsporeforming rods, the period of viability of its members in dried egg products may be underestimated. Results of Gibbons and Moore (15, 16), Wilson (42), and Ayres and Slosberg (5) attest to the fact that *Salmonellae* show considerable longevity in dried egg products. Recently, members of the

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group have been isolated from dried albumen that had been stored at room temperature for three years (4).

McCullough and Eisele (27, 28, 29, 30) not only have effectively demonstrated the production of experimental human salmonelloses by feeding human volunteers graduated dosages of known species isolated from market samples of spray-dried eggs, but also have established threshold levels for infection by these agents. Species included in their experiments were strains of *Salmonella meleagridis, S. anatum* (*anatis*), *S. newport, S. derby, S. bareilly,* and *S. pullorum.* They concluded that the number of organisms required to produce illness could conceivably occur in reconstituted egg.

According to the few quantitative results available, however, the numbers of organisms that have been recovered from dried egg products are much smaller than were required to establish gastroenteritis in McCullough and Eisele's experiments. This observation does not lessen the significance of *Salmonellae* in dried egg, since the conditions for use of these products often are such that an opportunity is afforded for bacterial multiplication when the egg is reconstituted.

The present limited quantitative information concerning the incidence of *Salmonellae* in egg products is due to the fact that processing laboratories may not be properly equipped or have personnel trained to perform the tests. Also, the methods for determining and counting these organisms are complex and time consuming.

Many of the technics now adopted for use with food products were originally developed for isolating and identifying pathogens in feces, sewage, and tissues of infected animals and may not be equally suited for recovering the organisms from dried egg. Also, when one considers that there may be a very small number of *Salmonellae,* no *Shigellae*, few gram positive organisms or other enteric bacteria, and that egg contains several antigrowth factors, these differences may be important.

Procedures presently recommended for the isolation of *Salmonellae* employ media which utilize the inhibitory and differential qualities of oxidation-reduction potentials, toxic salts and dyes, biochemical reactions, and antigenic analyses. Hinshaw and McNeil (21) stress that workers in this field familiarize themselves with the most recent advances in enrichment broths and differential media.

RECOVERY METHODS

Two methods have been employed for recovering *Salmonellae* from egg products. One involves "direct streaking" of the sample on a selective medium; the other uses some preliminary "enrichment" procedure and subsequent isolation of some of the progeny on a selective solid medium. For the latter the suspected sample usually is inoculated into a liquid medium, mixed, and allowed to incubate for a sufficient period of time to allow the *Salmonellae* to reach the M-concentration (maximum stationary phase). Then one or more loopfuls are streaked on a plating medium.

One of the requirements of the direct streaking method is that the product being examined should contain a microbial population of sufficient size that a loopful (approximately 0.01 ml.) will give a reliable estimate of the contamination.

Gibbons and Moore (16) reported that over 99 per cent of organisms of the genus *Salmonella* in liquid whole egg were killed during drying and that, of those which survived in experimentally infected whole egg, there was a 97 per cent reduction in the course of a three month period when the product was stored at 45°F. Ordinarily, the number of viable pathogens in the dried material has been found to be so small that customary plating procedures are of limited value. It follows, then, that unless the product is grossly contaminated, the small amount of material that would be transferred by loop might give an erroneous concept of the sanitary quality of the product.

The "spread" plate, a modification of the direct streaking method, might have limited application as a technic for detecting *Salmonellae* in samples wherein the count is expected to exceed one such organism per ml. Since from 0.1 ml. to 1.0 ml. of liquid is spread over the surface when this method is used, it is important that the agar surface be quite dry in order that all liquid is absorbed.

Ordinarily the recommended procedure for isolating *Salmonellae* involves the use of media which induce these organisms to multiply at a more rapid rate than other organisms that may be present. While this method is commonly referred to as an enrichment procedure, the broths employed differ considerably in their mode of action. For example, in tetrathionate broth only those organisms having the enzyme tetrathionase are able to reduce the tetrathionate to thiosulfate. Iodine solution is added aseptically, shortly before use, to the sterile base medium to properly poise the oxidation-reduction potential so that it favors the development of *Salmonellae*. On the other hand, Selenite-F medium is considered a satisfactory enrichment broth for members of the genus *Salmonella* because it has been found to be less toxic for these organisms than for coliforms and other common sewage contaminants.

Of the several broths recommended as enrichment media for isolating members of the *Salmonellae* from feces, urine, and infected tissue, Selenite-F and tetrathionate have been the two most generally used for studying dried egg products. Gibbons and Moore (15) utilized tetrathionate, while Schneider (37), Solowey, *et al.* (39), and Ayres (2, 3) employed Selenite-F. Schneider was unable to isolate *S. pullorum* from any of 61 dried egg products when he used tetrathionate for enrichment, but recovered these organisms from 15 with Selenite-F. Cantor and McFarlane (10) employed both broths for recovering *Salmonellae* from fresh eggs.

Haines, Elliot, and Tomlinson (31) indicated a preference for incubating dried egg overnight in ¼-strength Ringer solution rather than in tetrathionate broth; they did not test Selenite-F. It is possible that one of the reasons that the British workers obtained a larger proportion of

isolations from Ringer solution resulted from the larger amount of sample used; one-gram amounts of dried egg were enriched in tetrathionate broth while ten-gram portions were incubated in the Ringer solution. Hurley and Ayres (22), in studying the effect of Ringer solution on pure cultures of *Salmonellae* and of common contaminants, found that the solution was nonselective and that any organism capable of withstanding the bacteriostatic agents present in egg would grow well and complicate isolating procedures.

Banwart and Ayres (6), using known loads of organisms representing each of the immunologic groups, compared logarithmic growth curves of eight strains of *Salmonella* in Selenite-F, tetrathionate, Ruys' broth (34, 35), and nutrient broth, and concluded that growth rates in the enrichment broths were less satisfactory than in nutrient broth. In the case of Selenite-F there was a decrease in numbers of cells of *S. anatis* during the initial incubation period. There was marked inhibition of *S. paratyphi* in tetrathionate. Ruys' broth, a medium included because Ruys (35) considered it superior to Muller's tetrathionate (33) for isolating *Salmonellae,* was the least satisfactory of the group; all of the organisms tested exhibited an extended lag phase and an evident inhibition of growth. However, the incorporation of whole egg into the base medium reduced the inhibitory properties of the enrichment broths; this was especially true in the case of Ruys' medium where, apparently, the organisms reproduced as well as in nutrient broth after whole egg was added. There was still considerable inhibition of *S. paratyphi* in tetrathionate even after the addition of egg. Also, when tetrathionate brilliant green bile, the recovery medium in the combined enrichment method proposed by Kauffmann (24), was used, it was found to be more inhibitory for laboratory strains of *Salmonella* than either Selenite-F or tetrathionate.

SAMPLING

Several methods have been proposed for the routine handling of dried egg samples. In the method used by Gibbons and Moore (15), 13 grams of egg powder were ground with 117 ml. of tetrathionate in a sterile mortar with a small amount of sand, or mixed in a sterile screw-capped Waring blendor jar and then an appropriate series of aliquots was dispensed to check the survival of *Salmonellae* by the Most Probable Number (M.P.N) technic.

Schneider (37) weighed 5 grams of egg powder on sterile smooth paper, transferred this powder to a bottle containing 45 ml. of sterile water, and agitated in a mechanical shaker. Twenty-five ml. of this mixture was inoculated into 25 ml. of double strength Selenite-F and incubated for 18 to 24 hours at 37°C.

The test used by Solowey, *et al.* (39), consisted of introducing 25 and 50 ml. of a 1:10 dilution of egg powder (11 grams in 99 ml. saline) into 50 ml. of Selenite-F enrichment broth and incubating for 18 to 24 hours at 37°C. This procedure was modified (2) to give quantitative

information by introducing a series of weighed dried egg samples directly into tubes of Selenite-F and, after allowing sufficient time for the powder to rehydrate, thoroughly shaking the tubes and then incubating these for 16 to 20 hours at 37°C. Ultimately, those giving presumptive evidence of *Salmonella* contamination were used in determining an M.P.N. for the sample.

PLATING MEDIA

The use of a large number of selective agars has been suggested for the detection of *Salmonella*. There is considerable difference of opinion among workers regarding which of these is the best medium. Usually one or more of the following agars are selected: bismuth sulfite (WB), desoxycholate citrate (DC), desoxycholate citrate salicin sucrose (DC-SS), desoxycholate citrate lactose sucrose (DCLS), Salmonella-Shigella (SS), and Kauffmann's brilliant green (KBG). Most of these media were devised not only to permit isolation of *Salmonellae* but *Shigellae* as well. Consequently, some of the selectivity that a medium developed solely for isolating *Salmonellae* might attain is lost. Ordinarily those media which are highly selective inhibit not only extraneous organisms but also many of the more fastidious *Salmonellae.*

Some of the advantages and disadvantages of these different media are the following: Gibbons (14) found that *Salmonella pullorum* is often missed on SS agar but usually grows well on bismuth sulfite agar. Owing to the large number of late lactose-fermenting coliform organisms which grew as pale colonies on desoxycholate citrate, use of that medium made isolation of *Salmonellae* arduous for Haines, Elliot and Tomlinson (31). Also, these British workers cautioned that some *Salmonellae,* and particularly *S. oranienburg*, failed to give typical blackening of the colony and the metallic sheen on the surface of the bismuth sulfite agar that is characteristic of *Salmonella* colonies. They considered WB more effective than DCSS, although neither was considered suitable for the growth of *S. gallinarum* or *S. pullorum*. The quantitative use, both of WB and SS agars, was criticized (2, 3), since common contaminative species produced colonies on both of these which could be confused with the *Salmonellae.* DCLS was found to permit colony development of *S. typhimurium* amounting to less than 1 per cent of that provided by nutrient agar (6); also, this medium was found to permit development of *Proteus* colonies which were culturally indistinguishable from *Salmonellae* (4).

According to Broh-Kahn (9), growth at the end of 24 hours on plates of KBG agar, inoculated directly with heavy suspension of stools or other material containing *Salmonella,* is almost certain to be due to these organisms; presence or absence of growth on this medium at that time "constitutes the most reliable single differential test for presence of a *Salmonella* organism."

Ayres (2) proposed the use of KBG for isolating *Salmonellae* from dried egg products. He considered that production of a bright red area around suspected colonies reduced the danger of overlooking possible

Salmonellae. However, too heavy streaking from heavily contaminated enrichment broths sometimes resulted in growth of coliforms which so crowded the *Salmonellae* that they failed to produce the typical color transformation. Also, species of *Pseudomonas* and *Bacillus* produced changes which were superficially similar to those brought about by *Salmonella;* species of *Proteus* did not give trouble. Usually, close scrutiny of the colonies was sufficient to reveal identifying characteristics of contaminative forms. Generally, these appeared as conical, irregularly shaped, or spreading opaque colonies, having rough or irregular edges.

Banwart and Ayres (6) found brilliant green agar to be much less inhibitory than bismuth sulfite, desoxycholate citrate lactose sucrose, and Salmonella-Shigella, and compared favorably with nutrient agar for seven of eight laboratory strains of *Salmonellae* tested.

Many laboratories employ two or more plating media simultaneously, in order that their findings will give a more reliable estimate of the contaminating load. It is probable that differences in the number of positive results accruing from such practice are due, not only to the mode of action of the toxic or reducing agents in the medium, but also to sample variation.

Some question might be raised regarding the use of brilliant green as the only plating medium. Since the accepted procedure in determining the presence of suspected *Salmonellae* consists of testing only those colonies surrounded by a brilliant red zone and disregards the lactose or sucrose fermenting organisms which form a yellow-green zone, it is possible that some atypical forms of *Salmonella* may be among the discards.

PRESUMPTIVE TESTS

Formerly, after a microscopic examination had been made of suspected colonies growing on the plating agars, transfers were made to differential media. Now that polyvalent antisera have become generally available, further differentiation procedures have been restricted to those organisms which give positive serological tests. Ewing and Bruner (13) made an extensive study of the serological properties of suspected organisms isolated from over 24,000 fecal specimens from patients and food handlers and observed that none of the 2,634 isolates which failed to react in polyvalent antiserum were later proved to be *Salmonellae*. The efficacy of this antiserum for recognizing *Salmonellae* is considered sufficient that cultures which react are presumed to be possible members of that genus. On the other hand, antigens closely related to, or identical with, those of the *Salmonella* occasionally are found in other *Enterobacteriaceae* (8).

For those laboratories desiring biochemical differentiation of suspected colonies rather than serological reactions as presumptive evidence, transfer of a portion of the colony is made to Krumwiede triple sugar agar slants or to slants of triple sugar iron agar (or its equivalent, Kligler's iron agar with 1 per cent sucrose added). All slants showing an

acid butt and alkaline slant with or without the formation of H_2S after 20 to 24 hours incubation are considered as possible *Salmonellae*. Urease, indole, and citrate tests generally are made from transfers from positive triple sugar slants in order to eliminate cultures which are not biochemically characteristic of the genus. Rarely, indole positive as well as lactose and sucrose fermenting *Salmonellae* have been isolated. Also, many of the biochemical reactions characteristic of the genus *Salmonella* are indistinguishable from those of members of the genera *Proteus, Pseudomonas,* and *Paracolobactrum*. Hence, positive results from either of these two screening tests must be considered only as preliminary or presumptive evidence that the organisms under consideration are *Salmonellae*.

It is of paramount importance that those who wish to isolate and identify *Salmonellae* from dried egg products recognize some of the aberrations among the genera in the family to which the genus *Salmonella* belongs. Bergey's Manual (6th ed.) states:

> Although fermentation of lactose, sucrose and salicin, formation of indole, gelatin liquefaction and failure to produce gas have been described for organisms serologically belonging to Salmonella, the practical recognition of this genus and studies of its constituent species suggest that these be looked upon as exceptions which do not invalidate the biochemical definition of the genus.

and

> . . . . Serological definition of the limits of the genus is fraught with many practical and theoretical difficulties. Indeed, there is increasing evidence of antigenic affinities of varying degree between *Escherichia, Salmonella* and *Shigella*.

FURTHER TESTS

In order to rule out the possibility that organisms other than the *Salmonella* have been isolated, confirmation must be made of all colonies. Both biochemical and serological properties are considered. Antigenic analysis is made in accordance with the Kauffmann-White schema (25) of the somatic (O) antigens in order to place members of the genus in large serological groups. Urease, indole, and citrate tests are made from transfers from cultures giving agglutination in polyvalent serum. Also, motility, ability to produce H_2S, and action on carbohydrates such as glucose, maltose, lactose, sucrose, mannite, dulcitol, and salicin are determined.

Complete identification of individual types requires characterization not only of the somatic antigens but also of the flagellar (H) antigens. It is evident when one reads the decision of the Salmonella Subcommittee of the International Society for Microbiology (26) that:

> By international agreement, serologically related types are considered to belong to the Salmonella group even if their behavior differs from the above qualities (fermentation of lactose or sucrose, liquefaction of gelatin, or production of indole). No organism possessing aberrant cultural or biochemical properties is to be included in the Salmonella group unless it contains O and H antigens typical of the Salmonella group.

While this decision seems to be a necessary one from a taxonomical standpoint, the adoption of a complete testing program by egg processing plants would require trained personnel and might be prohibitively expensive.

A SUGGESTED METHODOLOGY

The following method is suggested as a rational procedure for dried-egg processing plants to use in order to presumptively identify *Salmonellae*:

QUALITATIVE TEST

1. Aseptically introduce 100 grams of dried egg product into 900 ml. of Selenite-F. Mix. After allowing 2 hours for the product to reconstitute, shake and incubate for 18 hours at 37°C.

2. Streak 2 or 3 loopfuls on brilliant green (BG) and on bismuth sulfite (WB) agar plates in such manner that separate colonies can develop. Incubate plates for 24 hours at 37°C. Examine for typical colonies. On BG agar select well-isolated translucent colonies having smooth, small, flat to slightly raised colonies surrounded by a bright red zone. On WB agar choose small, well-isolated, flat to slightly raised black to light green colonies; these may be surrounded by dark zones. WB plates which show no typical colonies should be incubated an additional 24 hours before being discarded as negative. From both media 3 or 4 of each type of suspicious colony should be examined.

3. Use polyvalent *Salmonella* antiserum to check colonies which have been determined microscopically to be pure cultures of gram negative rods.

Ordinarily these three steps will yield sufficient information to indicate that no *Salmonellae* are present in negative samples and that the organisms may be presumed to be present if they react with the antiserum. If desired, the possibility that the organisms in question are other than *Salmonellae* can be eliminated by running indole, urease, and citrate tests on each suspected culture.

QUANTITATIVE TEST

Often the egg processor desires to know the extent of contamination during various stages of the processing operation. For this purpose he would employ a quantitative procedure.

1. The dried material is measured aseptically in portions representing 0.1, 1, and 10 grams, and these are added to tubes or flasks containing 3, 9, and 90 ml. of Selenite-F enrichment broth respectively. For routine use, measuring cups or spoons facilitate the sampling procedure. Particle size, moisture content, and fat content vary among the several types of dried egg product; hence, it is necessary to calibrate measuring devices for each type of dried egg tested. It is recommended that five tubes be used for each of the serial dilutions.

2. Streak 2 or 3 loopfuls of growth from each tube on brilliant green

(BG) and on bismuth sulfite (WB) as outlined in the qualitative test.

3. Use polyvalent *Salmonella antiserum* to check colonies which have been determined microscopically to be pure cultures of gram negative rods.

4. Most Probable Numbers data may be obtained by comparing the number of plates (from which at least one of the suspected colonies produces positive agglutination reactions with polyvalent *Salmonella* antiserum) at each dilution with the corresponding number in probability tables computed for 100 ml. of water (1).

Ordinarily, this is as far as the plant would proceed. If desired, however, confirmation and even completion tests can be carried out in the orthodox manner by identifying somatic (O) and flagellar (H) antigens.

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THE OXIDATION OF WATER BY CERIUM (IV) PERCHLORATE

FREDERICK R. DUKE AND JOHN A. ANDEREGG

Institute for Atomic Research and Department of Chemistry
Iowa State College

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Cerium (IV) in perchloric acid solution is not completely stable, the following reaction taking place:

$$4\ Ce^{+4} + 2H_2O \rightarrow 4\ Ce^{+3} + 4H^+ + O_2.$$

The kinetics of the reaction have been studied by Kolp and Thomas (1) who conclude that thermally activated Ce (IV) dimers are involved in a homogeneous reaction. They further conclude that these activated dimers are deactivated upon collision with cerous ions leading to a kinetics expression:

$$\frac{-d\ \mathrm{Ce(IV)}}{dt} = \frac{-k\ [\mathrm{Ce(IV)}]^2}{[\mathrm{Ce(III)}]}.$$

The data upon which these conclusions are based are questionable in view of the large variation on the rate constant which they calculate. Further, the above investigators were able to interpret their data only at one temperature, the other temperatures yielding inconclusive results.

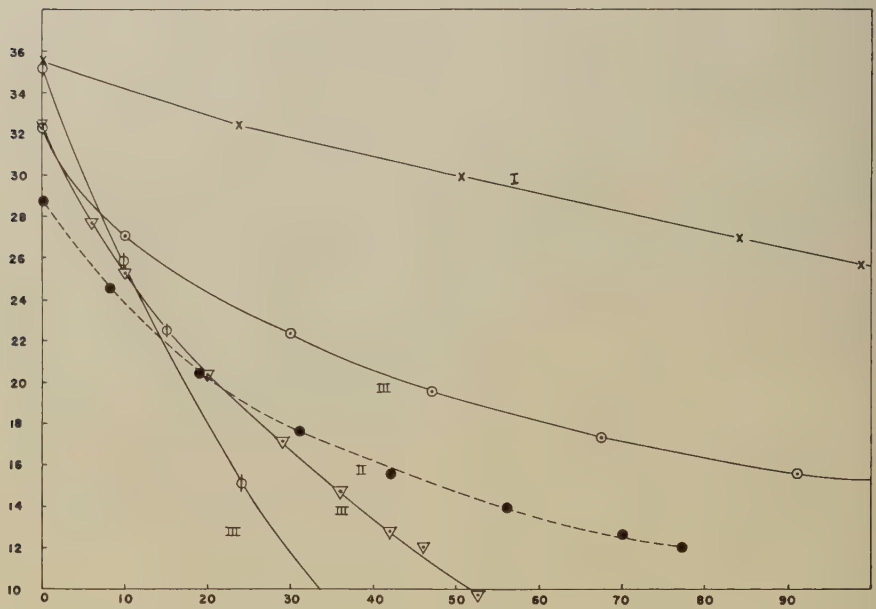
We have reinvestigated the kinetics of this reaction, and have found that, in all instances, it appears to be heterogeneous.

EXPERIMENTAL

Solutions of Ce (IV) perchlorate, prepared by electrolytic oxidation of Ce (III) perchlorate, were placed in a flask under a reflux condenser at an ionic strength of 4. The ionic strength was maintained by the addition of $HClO_4$ and $NaClO_4$, and by addition of $Al(ClO_4)_3$ and $Ce(ClO_4)_3$. Since the reaction is photosensitive the flask and condenser were wrapped in aluminum foil; the flask also had a layer of closely fitted asbestos surrounding it. The contents of the flask were heated to boiling and allowed to boil for about thirty minutes before samples were taken for analysis; this boiling period insured destruction of oxidizable impurities. During the sampling period, the heaters were turned down to maintain slow boiling and it was found that the temperature remained constant at 120° ± 2°C. Samples were taken by pipet at suitable intervals, quenched in KI solution and the I_2 titrated with 0.01 N thiosulfate.

RESULTS AND DISCUSSION

Plots of Ce (IV) vs. time are shown in Figure 1 for various mixtures of Ce (IV), Ce (III), aluminum, and hydrogen ions with added solid materials. Attempts to fit any of these curves with analytical expressions based upon a variety of homogeneous mechanisms failed. In general, this reaction is less than second order in cerium (IV) and something under negative second order in cerous and hydrogen ions. The experiments



I. ×: 0.2M Ce (III), 4.0M [H^+], glass beads
II. ●: 0.1M Ce (III), 4.0M [H^+], glass beads
III. ▽: 0.1M Ce (III), 3.0M [H^+], glass beads
· : 0.1M Ce (III), 3.0M [H^+], fused silica
⌽: 0.1M Ce (III), 3.0M [H^+], porous plate

FIGURE 1

were done in the presence of various solid materials such as chipped porous plate, silica gel, and glass wool. It was found that these materials all catalyzed the reaction. The mechanism which we propose is:

$$\mathrm{Ce\,(IV)} + \mathrm{Surface} \rightleftharpoons \mathrm{Adsorption}$$
$$\mathrm{Ce\,(IV)\ (adsorbed)} + H_2O \rightleftharpoons \mathrm{Ce\,(III)} + \mathrm{HO\,(adsorbed)} + H^+$$
$$2\ \mathrm{HO\,(adsorbed)} \rightarrow H_2O_2\ \text{(slow step)}$$
$$2\ \mathrm{Ce\,(IV)} + H_2O_2 \rightarrow 2H^+ + O_2 + 2\ \mathrm{Ce\,(III)}\ \text{(fast)}.$$

Under these conditions, the orders would depend upon the points

of equilibrium of the first two reactions, and the relative extents of adsorption of Ce (IV), Ce (III) and H^+. Thus, if the first equilibrium lies far to the left, one would expect to find second order in Ce (IV); if far to the right, zero order in Ce (IV). If, in the second equilibrium, the Ce (III) remained on the surface of the glass, any order between zero and minus two could be expected. The same sort of reasoning applies to the hydrogen ion. The results indicate that all three of these ions incompletely but detectably cover the surface of glass, porous plate, and silica, because the order in Ce (IV) is between 1.5 and 2, and the order in $[H^+]$ and Ce (III) is between negative 1.5 and 2. Thus, the most appropriate mechanism for this reaction appears to be heterogeneous and involving neutral hydroxyl radical.

LITERATURE CITED

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DIORYCTRIA DISCLUSA HEINRICH, N. SP. (PHYCITIDAE) AND ITS PARASITES IN IOWA[1]

MAURICE H. FARRIER[2] AND OSCAR E. TAUBER

Department of Zoology and Entomology
Iowa State College

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In June, 1946, larvae of *Dioryctria disclusa* Heinrich[3] were noted[4]

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[1] Journal Paper No. J–2256 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1102.

[2] Now on active duty with Medical Service, U. S. Army. Mailing address: Entomology Dept., North Carolina State Agricultural Experiment Station, Raleigh, North Carolina.

[3] Carl Heinrich, of the U. S. National Museum, identified the insect concerned and has generously suggested that the description be published as a part of this manuscript. *It should be understood that Mr. Heinrich is to be credited as the original describer.* His description of the new species is as follows:

"*Dioryctria disclusa,* new species, Carl Heinrich

"Maxillary palpus of male squamous.

"Fore wing smooth; basal area to antemedial line orange yellow; area beyond brownish red, more or less shaded or suffused with yellowish orange (on some specimens the ground color of the entire wing yellowish orange), usually the red shade most conspicuous in the area between subterminal line and termen; transverse lines narrow, white; a white streak along lower margin of cell between the transverse lines; antemedial line faint, oblique, nearly straight; subterminal line stronger, set well out, rather near terminal margin, outwardly angulate between veins 6 and 1b; discal mark (when distinguishable) a white line along discocellular vein; some very short white dashes on terminal margin; cilia smoky white. Hind wing smoky white to pale smoky gray, the paler examples showing a very faint ocherous tint; veins slightly darkened; cilia whitish.

Alar expanse, 24–29 mm.

Type. — U.S.N.M. No. 60925

Type locality. — Tryon, North Carolina

Food plants. — *Pinus* spp. Larvae feeding in the cones.

"Described from male type and 3 female paratypes from the type locality (15 June, 1903, and 3, 7, 16 June, 1904, Fiske); one female paratype from Raleigh, North Carolina (8 June, 1950, M. Farrier); one male and 2 female paratypes from Roosevelt, West Virginia (25 June, 1907, A. D. Hopkins No. 6404, reared from *Pinus virginiana*), one female paratype from District of Columbia (without date, Schoenborn); one female paratype from Lakehurst, New Jersey (25 July, 1931, F. Lemmer); and 3 male and 2 female paratypes from Ames, Iowa (20, 21, 23 June, 1946, M. Farrier). Several of these had been identified by Dyar as *auranticella* Grote.

"In appearance and genitalic structure *disclusa* is similar to *auranticella.* The latter is, on the average, a trifle larger and paler, and in distribution is a distinctly western species with the male maxillary palpus appreciably longer than that of *disclusa* and *in the form of an aigrette,* a type of palp not found on any other North American species. Dyar's *xanthaenobares* (Proc. Ent. Soc. Washington, vol. 13, p. 81, 1911) is a synonym of *auranticella.*

"Genitalia of all the American species of *Dioryctria* will be figured in a forthcoming revision of the Phycitidae."

(Authors' note: On May 26, 1951, additional specimens were collected by M. Farrier at Raleigh, N. C., as follows: One pupa, adult emerged June 7; one recently pupated, adult emerged June 10; four larvae, two reared to emergence on June 13 and June 14, respectively, and two preserved.)

[4] By H. M. Harris, State Entomologist of Iowa.

for the first time on the Iowa State College campus in cones of Scotch pine, *Pinus sylvestris.*

The widespread, though sparse, occurrence of *Dioryctria disclusa* in Iowa, and probably in other corn-growing states with stands of certain species of *Pinus,* may be of some importance, inasmuch as this lepidopteron may serve as alternate host for native, and, possibly, introduced parasites of the European corn borer, *Pyrausta nubilalis.*

The genus *Dioryctria* (Phycitidae, Lepidoptera) is Holarctic in distribution. Ragonot (1885) mentioned that the genus attacked Coniferae, and, to date, no exceptions have been found. McDunnough (1939) recognized eight species of *Dioryctria* in North America. Keen (1938) discussed damage by *D. xanthaenobares* Dyar and *D. abietella* D. and S. in western forests of the United States. Craighead (1949) listed *D. abietella, D. reniculella* Grote, and *D. amatella* Hulst as pests in eastern forests of the United States. Accounts of damage caused by different species, their life history, hosts, and control measures, as then known, were given by both Keen and Craighead. McKay (1943) studied the morphology, life history, hosts, and distribution of *D. reniculella* Grote and *D. abietella* D. and S. in Canada.

There evidently has been considerable confusion regarding the correct identity of various species of *Dioryctria.* The new species, *disclusa,* has been, in the past, sometimes considered as *D. auranticella* Grote. The latter, however, is distinctly a western species in the United States. Its most eastern records are from the sand hills of Nebraska where it was likely introduced in reforested areas on western pines, mostly *Pinus ponderosa* (Heinrich, 1950).

LIFE HISTORY AND HABITS ASSOCIATED WITH SCOTCH PINE CONES IN IOWA

Egg. Unfertilized eggs were deposited by captive females on July 14, 1947, and on July 17, 1948, inside glass vials. Eggs were arranged singly and randomly. Examination under a microscope showed no sculpturing or markings. Fresh eggs were white, elliptical, about 1 mm. long and approximately 0.75 mm. wide. No embryological development occurred. Apparently the ova are somewhat adhesive when first laid, inasmuch as some were found stuck on the upper as well as the lower curves of the vial. Under natural conditions the eggs are believed to be deposited directly onto cones.

Larva. The youngest larvae taken were found in pine cones on May 21, 1949. When compared with known stages of the related *Pyrausta nubilalis,* these cone larvae were considered to represent the second instar. Later stages were found until the latter part of June, when pupation occurs.

The larva characteristically enters the basal part of a cone, on the side nearest the twig to which the cone is attached. As the larva grows, the cavity in the cone is gradually enlarged. Reddish-brown frass, which darkens with age, is usually deposited in a web outside. The web is

fastened to the cone along with a few needles or also to the twig. Presence of entangled frass and of the undersized cone enables easy recognition of infested cones at some distance. In pitch pine cones some frass was often found inside the cone, in the cavity, after it had been sufficiently enlarged. Only second year cones are attacked. No other part of the tree was ever found injured.

Occasionally, two and even three larvae mature and pupate in one cone. Each larva occupies its own compartment, and apparently does not eat across into a neighbor's tunnel. In 1947, of a group of 93 cones examined, 13 contained two pupae or larvae, and one had three pupae. That year, infested cones were larger, at the time larvae were pupating, than during any of the three years the insect was observed on Scotch pine.

Small, dry, empty, damaged cones were found many times during the state-wide survey of 1949. These cones contained no larval or pupal remains and it thus seems likely that they were abandoned for a new food supply. This migration and the habit of younger larvae of wandering about outside in the web may partially explain the susceptibility of *Dioryctria disclusa* to a large and varied array of parasites.

Although the forthcoming Heinrich monograph will adequately present descriptions of the adults of *Dioryctria disclusa*, it will not contain diagnostic features for the larval stages. Therefore, the following is presented:

Description of Last Instar of *Dioryctria disclusa* Heinrich:[5]

Length, 17–27 mm.; maximum width, across fourth abdominal segment, 3.5–4.5 mm.; body tapering cephalad along thoracic segments, and rather sharply caudad from sixth abdominal segment.

General body color tawny buff above, darkening slightly toward each end, becoming ochraceous buff below; no gross lines or pigment patterns in skin proper; sclerotized pigmented pits present.

Head.—Head capsule 4.5 mm. wide. Basic color tawny with irregular lighter areas. Ocelli lighter than head capsule (Fig. 2, *A*). Mandible (Fig. 2, *F*) chestnut.

Thorax (Fig. 2, *A*).—Cervical shield tawny, fading anteriorly to light ochraceous buff, darkening to chestnut on posterior and lateral margins.

Thoracic spiracle with rim near central opening raised into ridge completely encircling opening, widening dorso-caudally. Central portion light, almost clear; rim chestnut. Prespiracular shield chestnut, darkening posteriorly to brownish-black.

Thoracic legs with simple claws, basal teeth absent. Tarsi with microscopic straight spines evenly distributed over lateral surfaces. Legs hazel to blackish-brown laterally, light ochraceous buff mesally.

Abdomen.—Sclerotized pigmented pits (Fig. 2, *A* and *B*), viewed microscopically, light brown and granular (Fig. 1, *A*).

Setal pattern similar to other phycitids. Seta iib on mesothorax with

[5] Sincere appreciation is due Hahn W. Capps, Division of Insect Identification, U.S.D.A., for technical advice.

pigmented sclerotized ringlike structure surrounding base; similar structure around base of seta iii on eighth abdominal segment. Pinaculae lighter than other body surface due to absence of pigmented granulation.

Anal shield (Fig. 2, *C*) sayal brown.

Abdominal spiracles with somewhat elliptoid center, long axis dorsoventrad; filaments, visible under high magnification, branching inward from rim.

Abdominal crochets (Fig. 2, *D*) biordinal. Anal crochets (Fig. 2, *E*) simple.

The above description is based on specimens preserved in 95 per cent alcohol after fixation in KAA solution. There was no apparent difference in pigmentation, after preservation for as long as a year, from that seen in fresh specimens.

The abdominal sclerotized pigmented pits are of particular impor-

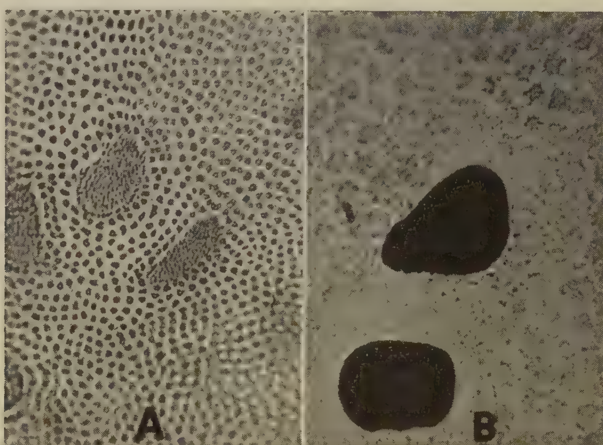

FIG. 1. Photomicrographs of sclerotized pigmented pits and surrounding micropits in larval skin of *Dioryctria disclusa* (*A*), and *D. amatella* (*B*).

tance for identification of some species of *Dioryctria*. Preliminary checking indicates that shape, sclerotization, arrangement, color of pits at muscle attachments, and size and shape of pigmented granules may furnish characters of use in a phylogenetic analysis of the genus, and perhaps of the family. The pits are not nearly as prominent, for example, in *D. disclusa* as in some other species. When seen under magnification, the pits of *disclusa* appear light brownish and granular, whereas those of *D. amatella* are very dark with the pigment granules seemingly fused into one mass (Fig. 1, *A* and *B*).

Pupa. Pupation was first noted as early as June 19, 1946; June 27, 1947; June 23, 1948; and June 12, 1949.

Pupae are usually found inside cones, but are occasionally outside

in the silken gallery. If within the cone, the larva spins a thin silken membrane across the entrance prior to pupation. The anterior end of the pupa is usually found toward this closure, with the larval head capsule and skin attached at the posterior end. No cocoon is spun.

An average length of pupation time for seven individuals at room temperature was 13 days. Shortest time was 10 days for two individuals; the longest was 16 days for one individual.

Adult. Adult emergence records for 1947 were obtained by placing infested cones in rearing boxes prior to, or during, pupation, and then daily counting the moths which entered the glass vials. About 400 cones

FIG. 2. Some morphological features of last instar of *Dioryctria disclusa; A,* Lateral view, head and anterior segments; *B,* Lateral view, abdominal segment; *C,* Anal shield, dorsal view; *D,* Abdominal crochet; *E,* Anal crochet; *F,* Mandible.

showing evidence of infestation were collected. A total of 278 moths emerged over the period from July 4 to July 29, with a peak of emergence about July 12. Many failures to emerge were due to parasitism of immature stages.

Adult emergence records for 1949 were obtained by isolating presumably infested cones in shell vials stoppered with wire screen. Adults were noted and removed on the day of emergence. From 138 cones only 67 moths emerged. Some of those not able to complete development

were parasitized, but others died in the pupal stage, for no apparent reason. The period of adult emergence in 1949 was from June 16 to July 4, with the peak on June 20. In both 1947 and 1949, peaks of emergence were toward the earlier part of the emergence period. The earlier emergence peak in 1947 is believed due to the "earlier" spring of that year.

The average length of adult life of seven moths kept in glass vials at room temperature in 1948 without food or water was 10 days; the shortest time was six days; and the longest was 15 days, for an unfertilized female which oviposited.

Flight is believed to occur mostly at night, and the moths are attracted to lights. Many specimens were seen in 1947 on the outside of lighted windows of buildings on the Iowa State College campus. Specimens have also been taken during the night in light traps.

Several experimental releases of adults were made in daylight in 1949. Moths set free at six to eight feet from Scotch pine would fly immediately to a tree. Generally they came to rest in the lower needles of a twig, or on a branch where they were difficult to see because of the blending of the moth's protective coloration with the color pattern of the bark. The moth came to rest with the body usually parallel to the branch, and the head toward the tip of the branch.

If released at 200 to 300 feet from any trees, moths usually started toward the nearest tree regardless of species. In many such releases, moths would fall to the grass before reaching a tree, indicating that *disclusa* is probably not a species with the habit of sustained flight or of mass migration for any long distance. After landing in grass, the moth walked out on the underside of a grass blade and hung upside down with the head toward the tip of the blade. Usually no attempt was made to fly farther under daylight conditions.

No striking differences in coloration of sexes was noted. No particular differences in coloration or size were noticed among adults reared from larvae infesting cones of various pines attacked.

Number of generations. Dioryctria disclusa has been observed in Iowa from May, when the larvae are first found, through late July, after the adults emerge. No second generation has been seen in cones of Scotch or jack pine, or in cones of any other conifers examined during a host survey. It seems likely that there is but one generation per year with the young larva overwintering, just as two other species of the genus are known to do, according to Keen (1938) and Craighead (1949).

Hosts. Cones of four *Pinus* species, Scotch pine, *P. sylvestris* L.; jack pine, *P. banksiana* Lamb.; red pine, *P. resinosa* Ait.; and pitch pine, *P. rigida* Mill., were found to be attacked by larvae of *Dioryctria disclusa* in Iowa.[6] No measurements were made of relative incidence of infestation of the four species of pines.

Distribution. Of Iowa's 99 counties, 61 were visited at least once

[6] One specimen, conspecific with the Iowa species, was reared from a loblolly (*P. taeda*) pine cone at Raleigh, North Carolina, June 8, 1950.

during the summer of 1949 in search of evidence of infestation by *Dioryctria disclusa.* Twenty-eight of the 61 counties were found to have had an infestation within their bounds during the spring of that year. Infestations were generally distributed over the state, although in eastern counties it was difficult to make observations because few Scotch pine were located along routes taken. Counties in which infestation was observed in 1949 are Adams, Audubon, Boone, Buchanan, Calhoun, Cass, Dallas, Fayette, Franklin, Guthrie, Keokuk, Linn, Lyon, Mahaska, Mitchell, Montgomery, Page, Plymouth, Polk, Ringgold, Sac, Story, Taylor, Union, Washington, Wayne, Winneshiek, and Worth.

Evidences of infestations prior to 1949 were found in Plymouth County (1945); in Tama County and Fayette County (1948); and in Story County (1939), according to internode counts.

Other evidence of the occurrence of *Dioryctria disclusa* in Iowa is shown by specimens deposited in the various insect collections of the state. One moth in the Iowa State Survey collection, Iowa Wesleyan College, at Mt. Pleasant, bears the following labels: Henry County, May 17, 1936; James Weir, collector; determined by A. E. Brower (Bar Harbor, Maine), in 1940. Four undetermined specimens were found in the Iowa State College collection with the notation — Ames, Iowa, June 18, July 10, and July 12, 1933; G. C. Decker, collector. In correspondence, Dr. Decker stated that he was unable to recall any additional details concerning these particular moths. This collection date of 1933 is not particularly surprising, since, as indicated above, cones infested about 1939 were found on jack pine in the Iowa State College Forestry Plots at Ames, Iowa.

Very careful surveys of the Iowa State College campus were conducted in 1947 and 1948 to determine the extent of infestation of Scotch pine. Of 178 Scotch pines examined in 1947, 74, or 41.6 per cent, were infested. In 1948 the number of trees was reduced to 163 by removal for construction. Only five of these, or 3.1 per cent, were found infested. It should be pointed out, however, that presence of cones on the tree is necessary for infestation. A check made on the campus in 1948 showed only 44.8 per cent of the Scotch pine were bearing at least one cone. Consequently, the percentages of infestation in 1947 and 1948 would actually have been nearly twice as high as given above when corrected for the factor of host-tree maturity. Casual observation in the spring of 1949 showed a greater infestation of campus trees than in 1948 but still much less than in 1947.

In 1949, infestation over the state was spotty. Iowa's plantings of Scotch pine are usually for windbreaks or landscape effects. Such plantings allow more cone-bearing branches to remain on the trees and thus provide a greater potential for cone production than on trees planted in blocks or seeded in natural stands. Greater incidence of infestation of isolated plantings can then be expected because of more plentiful host material. Jack pines in a forest reserve in southeastern Iowa had no observable infestation.

Economic significance. At present, *Dioryctria disclusa* is not an insect of economic importance in Iowa. It would become of importance as a pest only if natural reseeding[7] of pines attacked by it were necessary to maintain stands of these species, and if the population of the insect was high enough, year after year, to destroy much of the seed. Even with this possibility, it is doubtful whether this insect will ever become a forest pest of importance in Iowa, inasmuch as the present information indicates that the population fluctuation of *D. disclusa* from year to year is rather wide.

HYMENOPTERA ASSOCIATED WITH *DIORYCTRIA DISCLUSA*

The following compilation is based mainly on rearings from larvae and pupae of *D. disclusa* collected in 1947 and 1949. Additional information is based on unpublished records of the Iowa State Survey collections,[8] and on notes, where available, taken from the literature for each species. Except as noted, all of these Hymenoptera were identified by members of the Division of Insect Identification, U. S. Department of Agriculture.

Meteorus tetralophae Muesebeck, Braconidae. Fourteen specimens were taken in 1947; this was the largest number of one species of parasite collected in the three years of investigation. Pupae of *M. tetralophae* were usually found toward the entrance of the cone cavity, or even outside the cone close to the opening of the cavity. Dead or moribund host larvae were always found associated with the parasite pupae. These larvae, when observed under magnification, had a hole in the lateral body wall, usually in the sixth abdominal segment, through which the parasite larva had emerged prior to its pupation on the outside of the host's body.

The above information is the first addition to the biology of *M. tetralophae* since the original description (Muesebeck, 1927), and constitutes the first report from Iowa or anywhere in the western or middle-western United States.

Meteorus sp., Braconidae. Empty pupal cases identifiable only as *Meteorus* sp., from which the adults had emerged, were found in Mitchell, Polk, and Ringgold counties during the summer of 1949. In a number of instances remnants of a dried, shriveled phycitid larva were in the cavity with the empty pupal case of the parasite. The cases were probably from *Meteorus tetralophae.*

Apanteles bushnelli Muesebeck, Braconidae. One specimen emerged July 8, 1949. It had made its own cocoon on the inside wall of the cone cavity, after escaping from the larva of *D. disclusa.* Mueseback (1933) described this species from specimens reared from the pine tip moth,

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[7] Natural reseeding of Scotch pine has been found in Iowa. A new grove was set from seedlings taken from an old stand in Washington County. This is believed to be one of the first occurrences of reseeding in Iowa. Reseeding has been observed in eastern United States for some years (York and Littlefield, 1942).

[8] In charge of H. E. Jacques and D. D. Millspaugh, Iowa Wesleyan College, Mt. Pleasant.

Rhycionia frustrana var. *bushnelli* Busch., in Nebraska. The present notation is its first report from Iowa.

Bracon gelechiae Ashmead, Braconidae. Only one specimen was taken in 1947. Two more were collected in 1949, when it was found a primary parasite of *D. disclusa,* emerging from the larva and spinning its cocoon as described by Fiske (1903). Emergence date for both 1949 specimens was June 18. Insofar as known, this is the first specific report from Iowa, although *gelechiae's* general distribution has previously been given as over the entire United States (Muesebeck, 1925).

Scambus hispae (Harris), Ichneumonidae. Two pupae were taken in 1947. Dead larvae or pupae of *Dioryctria disclusa* were found in the cones with each parasite which was in a thin, silken, rather irregularly shaped, white cocoon attached to the wall of the cone cavity. Adult parasites emerged June 21, 1949. Beach (1892) previously reported this species from Iowa under the name *Pimpla indagatrix.* Townes (1944) listed three species of phycitids which have been reported as hosts for *Scambus hispae*, but the present record is the first report of its attack of any *Dioryctria.*

Calliephialtes comstockii (Cresson), Ichneumonidae. Five specimens were collected in July, 1947. Judging by its size, it is undoubtedly a solitary parasite of *Dioryctria disclusa.*

The above is the first report of this insect in Iowa, or in the entire Mississippi valley. Both of these areas are largely beyond the regions of natural coniferous forests to which Cushman (1927) believed its range was limited. Townes (1944) listed a "new species" of *Dioryctria* as being parasitized by *Calliephialtes comstockii.* This "new species" was mentioned by Barnes, whose unpublished data Taylor (1929) is said to have included in his work.

Coccygomimus aequalis (Provancher), Ichneumonidae. Four specimens were reared in 1947. Again, judging by its size, it is likely a solitary parasite of the host phycitid. Townes (1944) noted this species as present in nearly every state, including Iowa, with the exception of those in the northwest. He also noted it as a parasite of two species of phycitids, but neither of them in the genus *Dioryctria.*

Itoplectis conquisitor (Say), Ichneumonidae. This was one of the most common parasites of *Dioryctria disclusa* in 1947, when 13 specimens were collected. It was a solitary primary parasite, using the pupal case of its host for protection of itself. It was present again in 1949.

Beach (1895) previously reported *I. conquisitor* from Iowa. Eight different species of phycitids were reported by Townes (1944) as hosts of *I. conquisitor;* however, no species of *Dioryctria* is among them.

Mesostenus thoracicus Cresson, Ichneumonidae. Only one specimen was taken, in 1947. This insect may be a primary parasite of *disclusa,* inasmuch as *Townes* (1944) listed it as a parasite of six genera of phycitids, but none was *Dioryctria.*

The number of specimens in the Iowa State Survey collection tends to support Cushman's (1929) statement that *M. thoracicus* is the most

abundant mesostenine in its range. Nothing, however, has been published on its biology. This is the first report from Iowa.

Ichneumon brunneri Rohwer, Ichneumonidae. One pupa of *Dioryctria disclusa* was collected on February 22, 1949, in a cone from jack pine. It was hoped that the over-wintering stage of *D. disclusa* had been discovered, and the pupa was incubated to await emergence of the moth. However, on March 31, a female ichneumonid emerged. She was fed daily with sugar solution, by moistening the cotton wad which stoppered the vial, and lived until May 2. On April 23, 1949, another pupa of *D. disclusa* was taken under similar circumstances; from this host emerged a male of *I. brunneri* on May 5. One other specimen was taken from host material collected in Page County in July, 1949.

When the pupal case containing the above developing female of *I. brunneri* was first discovered, the inside of the case appeared to be completely filled, when viewed in strong transmitted light. However, when observed later, it appeared about half empty. This reduction in size of the contents is believed to be caused by the ejection of the meconium mass just prior to pupation of the parasite. Perhaps, then, *I. brunneri* overwinters as a solitary late larva or prepupa, using the host's pupal case of the previous spring for its protection. On this assumption, it must be that it has but one generation per year, at least on *disclusa*. All specimens of this parasite were checked by H. K. Townes, who, in 1944, cited no known host or biological data regarding this species, nor did he note its presence in Iowa.

Elasmus meteori Ashmead, Elasmidae. Two specimens emerged from a cocoon identified as *Meteorus* sp. by A. B. Gahan. This *Meterous* had emerged from a larva of *D. disclusa* and spun its cocoon inside the burrow in the cone in the same fashion as does *Meteorus tetralophae*. Later, on July 1 and 2, 1949, two adults of *Elasmus meteori* emerged from the primary parasite's cocoon. This confirms a previous report of hyperparasitism, via a species of *Meteorus*, by Ashmead (1898), when he described the species from specimens reared from *M. vulgaris*, a primary parasite of *Canarsia hammondi*.

Elasmus meteori has never been previously reported from Iowa.

Eupelmus cyaniceps var. *amicus* Girault, Eupelmidae. One specimen was taken in 1947. It may be a primary or secondary parasite of *D. disclusa*. This is believed to be the first report of this variety from Iowa.

Dibrachys cavus (Walker), Pteromalidae. The exact relationship of *D. cavus* to *Dioryctria disclusa* is obscure. A few adults of the parasite were found dead in rearing boxes in 1947, but the species did not reappear in 1949, when more precise observations on parasite emergence were conducted.

Dibrachys cavus has been reported a parasite of two hymenopterous species which have been found as parasites of *D. disclusa*, namely, *Microbracon gelechiae* and *Itoplectis conquisitor*. Also, it has been reported a parasite of a number of species of *Meteorus*, which is represented by *M. tetralophae* in the present investigation. *D. cavus*, or its

common synonym *Dibrachys boucheanus* Ratz., is usually reported with other parasites, without mentioning whether it was a hyperparasite or a direct parasite.

ECONOMIC SIGNIFICANCE OF PARASITES

Four of the hymenopterons associated with *Dioryctria disclusa* are known parasites of the European corn borer, namely, *Scambus hispae* (Harris), *Itoplectis conquisitor* (Say), *Coccygomimus aequalis* (Provancher), (Caffery and Worthley 1927); and *Bracon gelechiae* Ashmead, (Muesebeck 1925). These four parasites have all been reported previously from Iowa, or from more extensive regions that would presumably include Iowa, some under synonymous names (Muesebeck, *et al.*, 1951).

This common association of these parasites with *D. disclusa* and the European corn borer leads to an interesting speculation. In very local areas, as found in Iowa in 1947 and 1949, the population of *Dioryctria disclusa* might be large enough to serve as an alternate host of these four parasites of the European corn borer. However, so far, no level of parasite population has been found which could exert an effective control of the borer. Small populations of *D. disclusa,* however, might serve as foci from which the native parasites could spread into the borers.[9]

SUMMARY

Life history and habits of a pine cone infesting lepidopteron, *Dioryctria disclusa* Heinrich, new species, were studied in Iowa from 1946 through 1949, with a few notes in 1950; in 1947 and 1949 specimens were isolated for parasite emergence records.

D. disclusa evidently has been present in Iowa for at least 20 years, but is of very little economic importance, because its destruction of the seed-producing function of the cone is of no concern in self-seeding for the maintenance of the small stands of pine which have been established as windbreaks or as ornamental plantings in this state. However, parasite studies have uncovered an interesting mutual association among certain parasites attacking both *D. disclusa* and the European corn borer, *Pyrausta nubilalis.* This relationship may be of supplementary value in the biological control of the corn borer in that *D. disclusa,* which is virtually of no economic importance in Iowa, might serve as a natural reservoir of hymenopterous parasites which prey upon the corn borer. The small amount of host material available in Iowa to support *D. disclusa* is, of course, a limiting factor in the future biological balance among the hosts and parasites concerned in this complex.

Eggs of *D. disclusa* are described. It is likely that they are deposited

[9] A very limited number of European corn borer larvae was collected in Iowa during the 1950–51 winter to see if this shift of parasitism was taking place. Two collections were made, one in Page County and one in Polk County, in cornfields adjacent to Scotch pine groves which were found to have been heavily infested with *Dioryctria* during the previous spring. A special report from the European Corn Borer Research Laboratory, Ankeny, Iowa, showed no parasites recovered from a total of 55 borer larvae that were incubated.

directly onto the cone. Larvae attack the small, green, second-year cones of Scotch, jack, pitch, and red pine in the spring by excavating the interior of the cone. Frass is usually deposited in a silken gallery or web outside the cone. A description of the larval stage is presented for the first time.

Pupation takes place in June in the still-attached infested cones, or in the webbing outside. Moths emerge from about the middle of June to the latter part of July, depending on the season. There appears to be but one generation per year in Iowa; young larvae evidently overwinter. By consent of Carl Heinrich, the describer, the description of *Dioryctria disclusa* as a new species is included in this paper.

D. disclusa was found rather generally distributed over the state. Lightest infestation appeared in eastern sections of Iowa where little Scotch pine, the apparently favored host, is found.

In the parasite rearing investigation, twelve Hymenoptera were found associated with *Dioryctria disclusa.* Included are six ichneumonids: *Scambus hispae* (Harris), *Calliephialtes comstockii* (Cresson), *Coccygomimus aequalis* (Provancher), *Itoplectis conquisitor* (Say), *Mesostenus thoracicus* Cresson, and *Ichneumon brunneri* Rohwer. There were three braconids: *Bracon gelechiae* Ashmead, *Meteorus tetralophae* Muesebeck, and *Apanteles bushnelli* Muesebeck. Also collected were three species of other families: *Dibrachys cavus* (Walker), (Pteromalidae); *Eupelmus cyaniceps* var. *amicus* Girault, (Eupelmidae): and *Elasmus meteori* Ashmead (Elasmidae). Six of these were found as direct parasites, four as probably direct parasites, one as a hyperparasite, and one of uncertain relationship to *D. disclusa.* All are reported from this host for the first time.

The following insects are reported from the Middle West, including Iowa, for the first time: *Dioryctria disclusa, Calliephialtes comstockii,* and *Meteorus tetralophae.* Additional records specifically for Iowa for the first time are: *Apanteles bushnelli, Elasmus meteori, Bracon gelechiae, Mesostenus thoracicus, Ichneumon brunneri,* and *Eupelmus cyaniceps* var. *amicus.* First known biological notes are offered for *Dioryctria disclusa, Meteorus tetralophae, Apanteles bushnelli* and *Ichneumon brunneri.*

Scambus hispae, Itoplectis conquisitor, and *Meteorus tetralophae* for the first time are listed as parasites of a member of the genus *Dioryctria.*

Dioryctria disclusa is recorded as the first definitely known host for *Ichneumon brunneri.*

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NEW SPECIES OF MIRIDAE FROM MISSOURI (HEMIPTERA)

HARRY H. KNIGHT

Department of Zoology and Entomology
Iowa State College

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The Miridae known from Missouri have been recorded by Froeschner (1949); however, a few species remained unidentified. This material and a few specimens in the author's collection have been studied, resulting in the discovery of new species described in the present paper.

Plagiognathus albellus new species

Allied to *nigrolineatus* Kngt., and runs in the couplet with it in my key (Miridae of Illinois, 1941), but differs in being devoid of all black lines and dots, being uniformly pallid in color; also differs in the more sharply defined keel on ventral median line of the genital segment, with slight yet apparent differences found in the small claspers.

MALE: Length 3.7 mm., width 1.34 mm. Head: width .69 mm., vertex .30 mm., pale, eyes reddish brown. Rostrum, length 1.21 mm., just attaining tips of hind coxae, pallid, apex dusky. Antennae: segment I, length .25 mm.; II, 1.04 mm., cylindrical; III, .69 mm.; IV, .36 mm.; uniformly pallid. Pronotum: length .52 mm., width at base 1.08 mm. Dorsum clothed with recumbent and suberect, pale simple pubescence. Hemelytra uniformly pale, subtranslucent, membrane and veins pallid, no trace of fuscous color. Legs, including tibial spines, uniformly pallid, tarsi yellowish to dusky. Ventor, and in fact, the whole insect except eyes, uniformly pallid. Genital segment distinctive as stated above.

HOLOTYPE: ♂ June 2, 1944, St. Louis, Missouri (R. C. Froeschner); author's collection. Paratypes: ♂ June 2, ♂ June 16, 1944, St. Louis, Mo. (R. C. Froeschner).

Reuteroscopus froeschneri new species

Larger and darker colored than *ornatus* Reut., the dorsal surface broadly infuscated; without indication of a tubercle on dorsal wall of male genital segment; length of second antennal segment not equal to width of pronotum at base.

MALE: Length 3.5 mm., width 1.47 mm. Head: width .70 mm., vertex .39 mm., greenish yellow, frons and vertex fuscous. Rostrum, length 1.47 mm., reaching slightly beyond tips of hind coxae; yellowish brown, apex black. Antennae: segment I, length .23 mm., fuscous; II, .97 mm., cylindrical, clothed with fine short pubescence, uniformly fuscous; III, .69 mm., blackish; IV, .43 mm., blackish. Pronotum: length .65 mm., width at base .82 mm.; fuscous over a greenish-yellow back-

ground. Dorsum clothed with fuscous to black simple pubescence, intermixed with recumbent, silvery sericeous pubescence.

Hemelytra uniformly infuscated, corium slightly greenish on base, basal edge and tip of cuneus pale. Membrane uniformly deep fuscous, veins about smaller areole slightly paler, a pallid spot by tip of cuneus. Venter and thorax dusky greenish yellow. Legs pale to dusky, femora with double row of fuscous dots on anterior aspect. Genital segment with a sizeable membranous area above and anterior to the claspers, the sclerotized margins without tubercle.

FEMALE: Length 3.9 mm., width 1.68 mm. Head: width .70 mm., vertex .43 mm. Antennae: segment I, length .26 mm., fuscous; II, 1.12 mm., cylindrical, slightly more slender on basal half, yellowish brown, more fuscous on apical half, a narrow band at base, blackish; III, .77 mm., dark fuscous; IV, .38 mm., fuscous. Pronotum: length .69 mm., width at base 1.38 mm.; yellowish to brown, basal area of disk clouded with fuscous. Scutellum, clavus, and apical half of corium dark fuscous, margins of dark color not sharply defined as in *ornatus* Reut. Cuneus pale to yellowish, often fuscous on middle. Membrane deep fuscous as in male, pale spot by tip of cuneus more clear, also a pale half moon mark shows in submargin near middle.

HOLOTYPE: ♂ May 28, 1947, Galena, Missouri (R. C. Froeschner); author's collection. *Allotype*: taken with the holotype. *Paratypes*: 2 ♂, 2 ♀, taken with the types.

Ceratocapsus seticornis new species

Allied to *modestus* Uhler but distinguished from this and other known species by the prominent, erect, setigerous hairs on antennae and legs.

MALE: Length 4.9 mm., width 1.7 mm. Head: width .91 mm., vertex .35 mm.; eyes scarcely raised above contour of vertex and frons, provided with many short but distinct pubescent hairs; dark brown, paler on juga and about base of antennae. Rostrum, length 1.64 mm., reaching to middle of hind coxae, reddish brown, third segment paler. Antennae: segment I, length .52 mm., with several erect setose hairs, yellowish; II, 1.69 mm., tapering gradually from more slender at base to thickness at apex about equal to segment I, uniformly yellowish, rather thickly set with erect, setose yellow hairs, the length of most hairs equal to and a few exceeding width of segment; III, .65 mm., thickness nearly equal to segment II, dusky brown; IV, .56 mm., reddish brown; last two segments with fewer setose hairs. Pronotum: length .82 mm., width at base 1.35 mm., dark brown, moderately shining, disk as well as the scutellum, sparsely set with long, erect, setose hairs.

Hemelytra distinctly shining, dark fuscous brown, clavus paler brown and translucent, sparsely set with erect strong bristles and intermixed with sparsely set simple pubescent hairs, but no sericeous hairs evident. Cuneus darker than corium, a single row of bristles along inner margin. Membrane and veins rather uniformly infuscated. Legs brownish

yellow, shining, hind tibiae dark brown, clothed with erect strong bristles and hairs, length of bristles on tibiae equal to one and one-half times thickness of segment. Venter shining, dark brown; genital segment and claspers distinctive. Claspers quite similar in form to *modestus* Uhler, but basal dorsal prong with two small spines near apex, the middle prong of clasper more evenly curved, not angulated as in *modestus*.

HOLOTYPE: ♂ August 23, 1942, Holcomb, Missouri (R. C. Froeschner); author's collection.

Genus **Neolygus** Knight

Lygus (*Neolygus*) Knight, N. Y. (Cornell) Agr. Exp. Sta. Bull. 391: 561, 1917. Orthotype: *Lygus* (*Neolygus*) *communis* Knight.

Neolygus Knight, Bull. Ill. Nat. Hist. Surv. Div., vol. 22, Art. 1: 137, 1941.

Recent authors, hoping to simplify the classification of Miridae, have listed *Neolygus* as a subgenus of *Lygus* Hahn, as first described. In my revision of the genus *Lygus* (1917) I divided the genus into six groups, and trying to be conservative, refrained from naming all the subgenera which were actually indicated. However, one of these groups stood out far apart from all the others, so I ventured to erect *Neolygus* as a subgenus. During the next twenty years, spent in continued study of Miridae, including annual collecting and host plant studies of *Lygus* and *Neolygus* species, my convictions were strengthened that *Neolygus* stands apart as a good genus. When in the course of my work for the publication, *Miridae of Illinois* (1941), and following my long considered opinion, I elevated *Neolygus* to the status of genus. Few genera of Miridae have had so much study and as much evidence revealed on relationships as has *Neolygus*. Let us review the evidence: (1) In *Neolygus* the right genital clasper has a distinctive form, the terminal portion tapering to a sharp point, never with the short curved claw which is so characteristic of *L. pabulinus* Linn. and *L. pratensis* Linn. and other members of subgenus *Exolygus* Wagner. (2) The left clasper of *Neolygus* is likewise distinctive, right-angled at middle and provided with a prong; base of clasper never with the broad dorsal lobe or with dentate edges as in *Lygus* and *Exolygus*. (3) Slater (1950) has shown the taxonomic value of female genital structures in Miridae and states: "Indeed, the species of *Neolygus* studied do not appear to be closely related to the *Apolygus* complex, and their former position within the same genus appears to have been based upon a superficial resemblance rather than any true phylogenetic relationship." Slater's illustrations of female genital structures of *Lygus pabulinus* (L.) show wide differences from those of *Neolygus communis* Kngt. (4) *Neolygus* overwinters only in the egg stage in bark or cambium of trees and shrubs, hatches as the new leaves develop and has only one generation each year; while *Lygus pabulinus* (eggs and adults) and *Exolygus* species hibernate as adults, have two or more generations per year, where seasons are long enough, and the nymphs develop primarily on herbaceous plants. (5) *Neolygus* species have a

thin body integument, the cuticula impunctate; they can live and survive only where the humidity ranges around fifty per cent or higher; they are typically boreal in distribution, living on trees and shrubs, with a few species extending southward along valleys and water courses where the humidity is suitable.

The highly developed and specialized type of genital claspers indicates that species of *Neolygus* are among the most recent species, phylogenetically speaking. A majority of the species are found in eastern North America, where glacial ice has been widespread and the vegetation forced to migrate back and forth with the glacial periods. Their requirements for boreal conditions, of trees or shrubs and humidity, all go hand in hand with their present distribution.

Neolygus betulae new species

Runs to *alni* Kngt. in my key (Miridae of Illinois, 1941, p. 15), but distinguished by the pale green color without a bronze sheen, also more robust; differs in form of the genital claspers, apparently allied most closely to *atritylus* in these structures.

MALE: Length 5.9 mm., width 2.4 mm. Head: width 1.12 mm., vertex .43 mm. Rostrum, length 2.29 mm., just attaining apex of hind coxae, greenish, apex blackish. Antennae: segment I, length .65 mm., green; II, 2.03 mm., yellowish green, apex dusky; III, 1.04 mm., fuscous; IV, .60 mm., blackish. Pronotum: length 1.15 mm., width at base 1.94 mm.; greenish yellow, disk and the whole dorsum clothed with fine, recumbent, pallid pubescence. Hemelytra pallid translucent, embolium and cuneus distinctly green, a faint fuscous cloud on inner apical angles of corium, cuneus uniformly greenish translucent. Membrane pale, inner apical area and the larger areole may be clouded with fuscous, often clear. Venter and body uniformly pale yellowish, genital segment more green. Legs yellowish to green, unmarked; tibial spines brownish, with small fuscous dot at base of each spine. Genital claspers distinctive, rather similar to *alni* but the right clasper with terminal hook curved downward and approaching the small thumb at lower apex much more closely.

FEMALE: Length 5.8 mm., width 2.47 mm. Head: width 1.15 mm., vertex .45 mm. Antennae: segment I, length .56 mm., yellowish; II, 1.99 mm., greenish yellow; III, 1.08 mm., fuscous; IV, .60 mm., fuscous. Pronotum: length 1.25 mm., width at base 2.07 mm. Slightly more robust, but coloration and pubescence very similar to the male.

HOLOTYPE: ♂ June 15, 1947, Rosebud, Missouri (R. C. Froeschner), on *Betula nigra;* author's collection. Allotype: same data as type. Paratypes: 2 ♂, 1 ♀, taken with the types. 3 ♂, 4 ♀, June 8, 1944, Van Buren, Missouri (R. C. Froeschner). IOWA — 2 ♂, 1 ♀, July 13, 1927, Donnelson (H. M. Harris and H. G. Johnston), on birch (*Betula*).

Neolygus aesculi new species

In my key to *Neolygus* (Miridae of Illinois, 1941), this species runs to the couplet with *nyssae* Kngt., but may be distinguished by smaller

size, subtranslucent hemelytra, and red markings on head and sides of body.

FEMALE: Length 5 mm., width 2.42 mm. Head: width 1.04 mm., vertex .43 mm.; yellowish, frons with transverse red lines, tylus, juga, and lora chiefly red, eyes reddish brown. Rostrum, length 1.68 mm., just reaching to apex of middle coxae, yellowish, apex fuscous. Antennae: segment I, length .52 mm., yellowish brown; II, 1.68 mm., dusky brown, apex slightly darker; III, .95 mm., fuscous brown; IV, .60 mm., fuscous. Pronotum: length 1.08 mm., width at base 1.95 mm.; uniformly yellowish brown, calli showing hypodermal red granulations on anterior half, a few red dots about coxal cleft. Scutellum uniformly pale yellowish, mesoscutum showing some reddish.

Hemelytra pale, subtranslucent, pink tergites of abdomen visible, tip of embolium showing reddish coagulation in hypodermis. Cuneus translucent, outer base and apical margins with opaque coagulations. Membrane and veins clear, scarcely dusky on apical half. Dorsum clothed with very fine, recumbent, pale pubescence. Ventral surface pale to yellowish, sides of venter and episterna showing red granulations in hypodermis. Legs pallid to slightly yellowish, apical half of hind femore more yellowish and showing a slight amount of reddish in hypodermis; tibial spines yellowish, without dots at base.

HOLOTYPE: ♀ May 25, Kansas City, Missouri, on "buckeye leaves" (F. Rogers); author's collection. *Paratypes*: 2♀, taken with the type.

Polymerus froeschneri new species

Runs in the couplet with *unifasciatus* Fab. in my key (Miridae of Illinois, 1941, p. 166) but the rostrum relatively longer, reaching to apex of middle coxae; size much smaller.

MALE: Length 3.2 mm., width 1.5 mm. Head: width .86 mm., vertex .39 mm.; black, triangular spot each side of vertex and the lora, pale. Rostrum, length 1.17 mm., reaching to apex of middle coxae, dark brown to black. Antennae: segment I, length .34 mm., shining black, clothed with fine, brown, recumbent pubescence; II, 1.43 mm., cylindrical, slightly more slender toward base, yellowish brown to blackish, more black on base; III, .62 mm., black; IV, .56 mm., slightly more slender, black. Pronotum: length .73 mm., width at base 1.32 mm., black, moderately shining, basal margin narrowly and ventral margin more widely, pale to yellowish, top of collar brownish. Dorsum clothed with fine, pale to yellowish pubescence, clavus, scutellum, and ventral surface intermixed with sericeous recumbent pubescence.

Hemelytra blackish, moderately shining, basal angle of corium and apex bordering cuneus, tip of clavus, anal ridge, base and apex of cuneus, pale to yellowish. Membrane uniformly dark fuscous, veins about areoles pale. Venter and ventral surface or thorax, blackish. Legs black, tips of coxae and base of trochanter, band on basal half of femora, more widely on posterior pair, pallid; tibiae pale, a band just short of middle, the inner side on basal one-third, and apex, fuscous to black.

FEMALE: Length 3.4 mm., width 1.8 mm. Head: width .86 mm., vertex .43 mm. Antennae: segment I, length .30 mm.; II, 1.28 mm., distinctly more slender on basal half; III, .56 mm.; IV, .48 mm. Pronotum: length .82 mm., width at base 1.51 mm. Similar to the male in color and pubescence but body more robust.

HOLOTYPE: ♂ May 25, 1947, Kinsey, Missouri (R. C. Froeschner); author's collection. *Allotype*: collected with the type. Paratypes: 5 ♂, 2 ♀, collected with the types on the host plant, *Houstonia nigricans* (Lam.) Fern. 12 ♂ ♀ May 13, 1950, 34 ♂ ♀ August 19, 1951, Kinsey, Missouri, taken on the host plant at type locality by R. C. Froeschner. The species is named for Mr. Richard C. Froeschner who has done so much to make known the Hemiptera of Missouri.

Pachypeltocoris new genus

Runs to *Polymerus* in my key (Miridae of Illinois, 1941), but differs in the broad, compact body form, short antennal segments, and tumid scutellum. Eyes fitting closely against anterior margin of pronotum, covering anterior angles, but extending very little above the general contour formed by the frons and vertex; second antennal segment short, its length only slightly exceeding width of head across eyes. Pronotal disk and scutellum distinctly rugulose, but puncture not evident, disk of scutellum strongly convex, more tumid on apical half. Wing membrane dark but marked with numerous white dots. Arolia erect, divergent on apical half as in *Polymerus;* dorsum clothed with short fine pubescence and intermixed with closely appressed sericeous pubescence, more abundant on clavus and corium. Female with very long ovipositor, its base hidden by the overlapping hind coxae.

Type of genus: *Pachypeltocoris conspersus* n. sp.

Pachypeltocoris conspersus new species

Distinguished by the small, compact, robust form, tumid scutellum, and numerous white dots in the wing membrane.

FEMALE: Length 3.3 mm., width 2.16 mm. Head: width 1.10 mm., vertex .51 mm.; frons moderately convex, contour forming a uniform arc from base of vertex to middle of tylus, basal carina scarcely evident. Rostrum, length 1.60 mm., extending beyond hind coxae to near middle of abdomen. Antennae: segment I, length .32 mm., brownish black, ventral surface and line on dorsal surface, white; II, 1.17 mm., slender, slightly thicker on apical half but scarcely attaining thickness of segment I, dark brown, a pale band with proximal edge beginning at middle of segment; III, .41 mm., blackish, narrow apex pale; IV, .47 mm., brownish black. Head brownish black, more black on tylus, vertex, median line and sides of frons, spotted and marked with cream; juga and lora creamy white, juga with line on middle, and margins of lora blackish; gula and bucculae creamy white. Pronotum: length .83 mm., width at base 1.62 mm., basal margin sinuate at middle, lateral margins distinct but not sharp, a calloused line marking edge of propleura; distinctly trans-

versely rugulose but punctures not evident; calli distinct, slightly convex on anterior half, impunctuate, area between calli and before finely regulose, impunctuate; collar evident, narrow, visible only where vertex is in contact. Propleura rugulose, with pale spots which appear smooth and slightly elevated; coxal cleft distinct. Color yellowish brown to black, pronotal disk yellowish, irregularly darkened with brownish to black. Scutellum sharply convex, apical half strongly tumid, transversely regulose, disk brownish black, apex and basal angles yellowish, the blackish color invaded around edges by yellowish spots. Dorsum clothed with short, closely appressed, sericeous, silvery to golden pubescence, sparsely intermixed with short simple pubescence; sericeous pubescence thicker on clavus and corium.

Hemelytra with embolar margins arcuate, cuneus sharply deflected, almost vertical in position; membrane dark fuscous, dotted with some thirty white spots, veins and areoles as in *Polymerus*; yellowish to brownish black, basal angle of corium, spots on embolium and middle area of cuneus, yellowish. Legs: coxae and trochanters pallid, coxae with one or two irregular dark marks; front and middle femora with spots confined largely to ventral surface; tibiae dark reddish brown, interspersed with numerous cream spots and marks, spines short, black; tarsi blackish, middle segment chiefly pale. Venter brownish black, thickly and quite evenly dotted with creamy white. Ovipositor valves unusually long, extending from second ventral segment to tip of abdomen.

HOLOTYPE: ♀ May 31, 1943, Des Arcs, Missouri (R. C. Froeschner); author's collection.

Phytocoris tucki new species

Allied to *corticevivens* Kngt., and most likely lives on bark, as that species does; distinguished by antennal color and markings, second segment uniformly pallid gray; front tibiae with four pallid bands; genital claspers distinctive, right clasper truncated on apex; female brachypterous.

MALE: Length 7.5 mm., width 2.2 mm. Head: width 1.08 mm., vertex .43 mm., pallid, frons with several transverse to oblique brown lines, collum with two transverse marks each side of middle, a heavy mark behind lower half of eye, opposite the stripe crossing coxal cleft; tylus with vitta on apical half, lorum and buccula with dark line on dorsal margins. Rostrum, length 3.55 mm., reaching to middle of venter, pale, apical half brown. Antennae: segment I, 1.73 mm., reddish brown, color broken by small and large white spots, more white on middle; beset with several white bristles, but their length not exceeding diameter of segment; II, 3.55 mm., pallid gray, more dusky toward apex, slender, finely pubescent, the color in this case very distinctive; III, 2.5 mm., slender, dusky brown, paler on base and fuscous toward apex; IV, 1.68 mm., fuscous. Pronotum: length 1.04 mm., width at base 1.73 mm., pallid to brownish, narrow basal margin white, submargin brownish shading to paler on middle; propleura pallid, a strong fuscous stripe passing through coxal

cleft and extending upon head behind eye, a second but finer stripe along dorsal margin; disk and hemelytra clothed with short erect blackish hairs and intermixed with recumbent, silvery pubescence. Scutellum pale yellowish, disk with small fuscous patches and dots, a darker patch on each side before the pallid apex.

Hemelytra elongate, pallid to dusky, marked with numerous dots and patches of fuscous and brown; membrane pale, rather evenly sprinkled with numerous fuscous dots which in places aggregate into patches, veins dusky brown. Legs pallid, marked with alternating bands of white and reddish brown, femora more strongly marked on apical half, the dark color invaded and spotted with pallid, hind pair with distinct oblique annulus on apical half; front tibiae with four pallid bands separated by narrower dark brown bands, a dark band on apex and one at base; similar bands on middle tibiae but the dark bands more broken by pallid spots; hind tibiae with dark bands less distinct; tibiae set with brown spines which in length about equal width of tibiae, but hind pair longer and tapering to more slender on apical half where spines may exceed width of tibia; tarsi yellowish, base and apex blackish. Venter fuscous to brown, sides darker brown, ventral area with pallid spots and merging to paler on middle; genital claspers distinctive, a prominent tubercle above base of left clasper, a smaller tubercle before base of right clasper; right clasper subrectangular, narrowed somewhat at middle, apex broadly truncate, about as wide as at base.

FEMALE: Length 5.84 mm., width 2.68 mm. Head: width 1.10 mm., vertex .52 mm.; pallid, frons with seven transverse to oblique brown lines, extending to median line but not uniting, collum with two transverse marks each side of middle; other marks as in the male. Rostrum, length 3.77 mm., reaching to base of ovipositor, pale, apical half brown. Antennae: segment I, length 2.34 mm., reddish brown, broken and spotted with several small and large white spots, two large spots on middle almost forming annuli, beset with several white bristles but their length scarcely equal to diameter of segment; II, 4.3 mm., pallid, becoming slightly dusky near apex, slender, finely pubescent, the color in this case very distinctive; III, 2.38 mm., slender, dusky brown, paler on base and fuscous toward apex; IV 1.65 mm., fuscous. Pronotum: length .91 mm., width at base 1.43 mm., shaded and marked with fuscous; calli pale, outer half with dark spot and smaller marks, narrow basal margin of disk white, six small fuscous marks bordering this anteriorly; propleura as in male. Scutellum pallid, a fuscous spot each side before apex and smaller dots in between.

Hemelytra abbreviated, cuneus rounded inwardly, membrane reduced to small flaps on inner margin, but enough remains to show sprinkled fuscous dots; cuneus chiefly pallid, inner margin at tip of paracuneus with prominent black spot, also marks on outer margin which appear as a continuation of a series of rectangular spots on embolar margin; clavus and corium with numerous dots and patches of fuscous, more abundant and consolidated on apical area of corium. Legs similar

to the male, tibiae annulated with white and brown. Venter broad and heavy, exposing two segments beyond tip of cuneus, sides marked and shaded with fuscous and brown.

HOLOTYPE: ♂ November 13, 1936, Marshfield, Missouri (Joseph B. Tuck); author's collection. *Allotype*: ♀ October 26, 1941, Freeburg, Missouri (R. C. Froeschner). These records indicate the species must have two broods each year; most likely it is a predacious, bark-inhabiting species as are its nearest allies. The species is named in honor of Joseph B. Tuck, one of my former students and fellow Missourian.

Phytocoris osage new species

Runs in my key to Phytocoris (Miridae Illinois, 1941) to group I, and to couplet with *breviusculus* Reut., but differs from this and others in the very long first antennal segment which in length greatly exceeds the width of head. Resembles *conspicuus* Johnston in color markings but the first antennal segment is much longer.

MALE: Length 6 mm., width 1.73 mm. Head: width .90 mm., vertex .39 mm., pale yellowish with orange marks on vertex, collum, and dorsal edge of lorae. Rostrum, length 2.98 mm., reaching to middle of 8th ventral segment, yellowish, apex blackish. Antennae: segment I, length 1.52 mm., slender, reddish orange, middle one-third pallid, the orange base with five or six pallid spots, apical portion with two or three spots, basal half with a few erect pale bristles; II, 2.81 mm., yellowish, basal one-fourth paler, also slightly paler on base of apical half; III, 1.82 mm., dusky yellow; IV, .99 mm., fuscous. Pronotum: length .86 mm., width at base 1.47 mm.; pale yellowish, disk with lateral submarginal stripe, joining at basal angles with a narrower submarginal basal stripe; a spot behind each callus, two stripes on collar and extending posteriorly to inner angles of calli, a stripe across top of coxal cleft, orange colored. Scutellum yellowish, without distinct marking. Pronotal disk and collar with several prominent, erect pale bristles.

Hemelytra pale to yellowish, marked with orange and fuscous; sparsely clothed with erect fuscous hairs, intermixed with recumbent, silvery sericeous pubescence; clavus with orange, claval suture pallid, bordered each side by fuscous line; corium pallid, apical area shaded with orange and fuscous, inner angle deeper fuscous but margined with roseus; paracuneus distinctly roseus, a tuft of black bristles on edge of areole; cuneus yellowish, more orange on apex; membrane with fuscous color in sprinkled pattern, lateral margin behind cuneus and spot at middle of margin, white, central area with very few dark specks. Legs slender, femora with reddish and fuscous, the dark color broken by several large and small pallid spots, dusky orange beneath; tibia pallid to dusky, front pair with three orange bands, annuli indistinct on middle pair; while hind pair have one broad band near base; tibial spines pallid. Venter yellowish, sides more orange. Genital claspers distinctive, right clasper rather slender, tapering to a slender point, without claw.

FEMALE: Length 5.0 mm., width 1.82 mm. Head: width .87 mm.,

vertex .47 mm. Antennae: segment I, 1.73 mm.; II, 3.20 mm.; III, 2.07 mm.; IV, 1.60 mm. Pronotum: length .73 mm., width at base 1.08 mm. Hemelytra abbreviated, cuneus rounded on outer margin, shorter than in male; membrane reduced to a small flap, three abdominal segments visible from above.

HOLOTYPE: ♂ September 18, 1942, Lanagan, Missouri (R. C. Froeschner); author's collection. *Allotype*: taken with the type. *Paratypes*: ♂, taken with the types. MISSOURI — ♂ June 27, 1942, Noel; ♂ June 29, 1941, Bixby; ♀ September 4, 1938, St. Genevive Co.; all collected by R. C. Froeschner. Named for the Osage Indians, original inhabitants of the Ozark plateau before the coming of the white settlers.

Horcias illini Knight

Bull. Ill. Nat. Hist. Surv. Div. Vol. 22, Art. I, p. 172, 1941, frontispiece illus.

Until recently this interesting species was known only from the type locality, Dongola, Illinois. Now we have identified a specimen collected by Wilfred S. Craig, May 13, 1939, near Columbia, Missouri.

Lygus rubroclarus Knight

Lygus vanduzeei var. *rubroclarus* Knight. N. Y. (Cornell) Agr. Expt. Sta., Bull. 391:567, 1917.

I now recognize this as a good species.

Lygus rubrosignatus Knight

Conn. Geol. Nat. Hist. Surv., Bull. 34:576, 1923.

Lygus pratensis var. *rubrosignatus* Knight.

Originally described as a variety, I now recognize it as a species.

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A PRELIMINARY STUDY OF THE DISEASES OF CORN AND SOME RELATED HOSTS IN GUATEMALA

IRVING E. MELHUS

Iowa State College — Guatemala Tropical Research Center
Antigua, Guatemala

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Diseases of corn are little known in Guatemala, although they destroy annually from 5 to 20 per cent of the crop. Semeniuk and Wallin (14) called attention to the influence of climate on four leaf parasites of corn in 1946. In 1949 (11) Muller reported a survey of corn diseases and the same year Melhus, Semeniuk, and Vestal (8) described the distribution and destructiveness of *Helminthosporium* leaf blight and the occurrence of resistant varieties of corn. Melhus and Smith (7) have studied *Helminthosporium turcicum,* pointing out the seriousness of this pathogen in certain regions where the rainfall is high, and the isolating of seven resistant varieties belonging to the giganteum group. The numerous climates, incident to seasons (wet and dry), wide range in rainfall (15 to 200 inches), and altitude differences (sea level to 9000 ft.) provide environmental conditions annually that permit most plant pathogens known to corn to develop (6). This wide range in climates and the diversity inherent in the corn not only influence the prevalence and destructiveness of the pathogens, but also afford a splendid place to search for disease resistant strains.

In order to explore the corns for disease resistance with some understanding, it became necessary to know what diseases were present and to observe the reaction of the causal agents to the wide range of varieties in the diversified climates. It is proposed to describe observations and data relating to the plant pathogens found on corn in Guatemala, 1944 to 1952 inclusive.

SMUT DISEASES

Corn smut caused by Ustilago zeae (Beckm) Unger. It is interesting that little corn smut has been found on corn in Guatemala, either in the highlands or lowlands during the past eight years. In our plots, in 1946, in six different climatic zones ranging in altitude from 150 feet to 8133 feet, only a trace to one per cent of smut developed, despite the fact that, in all probability, the land had grown corn at one time or another for over 5000 years. Likewise, the fields in the vicinity of these experimental sites showed no more smut than the corn in our trials. In 1951, when head smut caused serious losses in our plots at Antigua, there was only a trace of infection by *U. zeae.* Stadelman (15) and Muller (11) also reported little occurrence of corn smut in Guatemala.

Based on our experiences in Iowa, where smut is sometimes destructive and where continuous cropping to corn often increases the amount of smut present, one might expect that here in Guatemala, where corn follows corn year after year, often two crops per year, the incidence of and the damage due to corn smut would be very great. Temperatures in some climates in Guatemala are not too different from the growing season temperatures in some parts of the middle west, where smut is very prevalent. It is true that cultural practices are drastically different in the two countries. In the United States the corn stalks are plowed under or allowed to rot on the surface when the land is planted to oats or other small grain crops; in Guatemala, hoe culture prevails, and it is common practice to remove or burn all crop refuse before the new crop is planted, thus a considerable amount of inoculum may be destroyed. However, the crop is often left standing in the field for thirty to ninety days after maturity to allow it to dry, thus affording ample opportunity for the spores to reach the soil. In only one region have we ever seen more than a trace of corn smut. This was in 1945, around the pueblo of Todos Santos in the Department of Huehuetenango, at an elevation ranging from 7000 to 9000 feet (Fig. 1).

It may be that the long dry season of six months, coupled with the low humus content of the soil, influences the viability of the spores of *U. zeae*. The existence of little smut in Guatemala is not due to the resistance of the Guatemalan corns, as attested by the fact that many Guatemalan corns when grown in Iowa became heavily infected with corn smut. This was especially true on our experimental plots at Ames, Iowa, in 1945, 1946, and 1952, and in laboratory and green house trials (16).

Head smut of corn caused by Sphacelotheca reiliana (Kuehn) Clint. The life history of this smut is somewhat different from that of the corn smut organism, *Ustilago zeae*. The spores fall into the soil where they remain alive and infect the corn seedling before it emerges from the ground. Infection of the seedling before emergence results in a systematic distribution of the mycelium in the apical primordial tissues, where it remains until the development of the floral structures. Sporulation takes place only in the tassels and the ears. The ears that become infected enlarge and are greater in diameter and shorter in length than the normal ears (Fig. 2). Unlike *U. zeae*, head smut completely destroys the entire infected ear. When the ears mature the husks may rupture, exposing the black powdery mass within. Marked hypertrophy occurs in the floral parts of the tassel as shown in Figure 2. The parasitized tassel may become so heavy that it hangs over or breaks off. The same organism, *S. reiliana* occurs on teosinte (Fig. 3).

Previous to the summer of 1951, head smut had never been known to have caused serious damage to corn in Guatemala. In the plots at Antigua, in 1951, head smut was observed to be so prevalent as to indicate the advisability of carefully evaluating its effect upon the corn crop. Accordingly, five rows were taken at random from a field planted in March. Of 2214 plants observed, 274 or 12.38 per cent were found to have

infected tassels. Of these 274 plants, 242 or 88.32 per cent had infected ears, and eleven plants without infected tassels were found to have infected ears. On the basis of one ear per stalk, 11.43 per cent of the total ears were destroyed by head smut in this particular field.

Most of the infection occurred on the short to medium plants and was rarely found on the tall ones, suggesting dwarfing effect, as well as a destruction of the ears and tassels.

FIG. 1. Corn smut on ears of corn. The smut fungus induces marked hypertrophy of the kernels. These swollen tissues disintegrate, releasing masses of black spores.

Several single crosses and inbreds from the United States, and open pollinated varieties from Mexico, Puerto Rico, and Guatemala were evaluated for smut infection. The plantings made in April were relatively free of head smut, while those made in February and March ranged from 4.68 to 18.37 per cent infected plants. Again the disease was observed to be most prevalent on the short plants. The comparatively low incidence of infection in the April plantings, just previous to the beginning of the

rainy season, suggests that the germination of the chlamydospores and infection were not favored by the high moisture content of the soil incident to the rainy season.

Tripsacum smut caused by Ustilago dieteliana. In the fall of 1946 this smut was found near Coban on a planting of *Tripsacum latifolium.*

Fig. 2. Head smut of corn. The pathogen invades the young ear shoots and tassels. Marked hypertrophy of the growing reproductive tissues takes place. Later the distorted tissues are a black powdery mass of spores.

This smut did not occur on the crown leaves, but only on the leaves and flowers of the stems. No galls were formed as in corn smut, instead, the parenchymatous tissues between the veins were destroyed. The long, pendant leaves of the stems, six to twelve feet tall, were green, with long black stripes. They were curled and rolled at the ends and their move-

ment in the wind afforded a strange sight (Fig. 4). This planting of *Tripsacum* comprised about an acre and the stand was comparatively uniform. The owner of the field said that he bought the plants four years previously from an Indian farmer who had been growing it as forage for about 15 years. The Indian farmer grew the *Tripsacum* between rows of sugar cane and other crops. Don Augusto Helmerick, the owner of the land, said he had never obtained seed from these plants, which was quite understandable in view of the fact that the smut was general in almost every plant.

The *Tripsacum latifolium* plants at Coban have been under observation for the past five years. Each season when the plants developed new stems the smut appeared. This suggested that the organism might be

FIG. 3. Head smut of corn on spikelets of teosinte.

systemic in the crowns of the plants. In order to test this assumption, portions of five crowns were dug and transferred to the Antigua trial grounds. The crown leaves were cut away, leaving only the terminal bud. All the plants grew. When the stems developed, the smut appeared in the same way as it did at Coban. This transplant experiment supports the observational evidence that the smut fungus existed in the growing points of the crowns of *Tripsacum*. In 1951 another field of about two acres was found infected on a finca southeast of Guatemala City. Here again the leaves were diseased. The same smut was collected in Mexico in 1945 on *Tripsacum* sp., about eleven kilometers out of Mexico on the road to Cuernavaca (altitude about 7500 feet). In this case the smut was evident only in the floral organs. The smut was not apparent on the

leaves of the stems, but it was observed that all of the branches bearing female spikes were infected, suggesting that the pathogen was systemic in part of the host.

T. latifolium is the most common species of *Tripsacum* in the highlands in Guatemala. *T. dactyloides* also occurs, but it is not as prevalent as the former species. *T. laxum* is common in the northern part of Guatemala at lower altitudes, in marshy, wet places in the departments of Alta

FIG. 4. A systemic smut of *Tripsacum latifolium* caused by *Ustilago dieteliana.* The leaves show long, black sori causing the leaves to become distorted and shredded. The flower stalk was destroyed. Collected at Coban in 1946.

Verapaz and Izabal. This species often forms a dense stand consisting of long, creeping, semiprostrate leaf stalks. These prostrate stems develop roots at the nodes eight to ten nodes above the crown which may form new clones. *T. laxum* has never been observed in flower, either in its native habitat or in the trial grounds at Antigua. Dense growths of *Tripsacum* occur along the railroad between Bananera and Puerto Barrios and also about eleven miles north of Coban at an elevation of 1000 feet on a finca owned by William Dieseldorf. However, *T. latifolium* is the only species on which the smut has been observed.

Cross inoculation studies using corn, teosinte, and Tripsacum smuts. The smuts were colected on corn, teosinte, and species of *Tripsacum*. The smut species were: *Ustilago zeae, Sphacelopheca reilinana,* and *Ustilago dieteliana.* The spore measurements were as follows: corn smut 6.0 and 12.0 microns, averaging 8.5; *Tripsacum* smut 9.0 to 13.5, averaging 11.0; and teosinte smut 10.5 to 13.5, averaging 11.7.

The chlamydospores of the three smuts were germinated on two per cent water agar. No measurements were taken of the sporidia, but those of the corn smut were longer than those of the teosinte and *Tripsacum* smuts. The sporidia of the teosinte smut were more yeastlike, i.e., shorter and wider than the corn or *Tripsacum* smut sporidia.

Monosporidial isolates were made from the corn and *Tripsacum* smuts, from cultures on potato-dextrose agar. In preparing the inoculum for cross inoculation, the sporidia were grown in liquid carrot decoction.

Two hundred and forty seedlings of sweet corn, variety Golden Bantam, were inoculated as follows: Lots of 20 seedlings each were hypodermically inoculated with each of five sporidial isolates of corn smut, five of teosinte smut, and two of *Tripsacum* smut. Seven days after inoculation the number of galled plants was counted. All of the sporidial isolates of corn smut produced galls. The two *Tripsacum* smut isolates produced galls on one and three plants, respectively. None of the seedlings inoculated with the teosinte smut isolates were galled, indicating that the teosinte smut was not pathogenic to corn by the method employed.

In a second experiment in the greenhouse, seedlings of sweet corn and teosinte (Chalco, Mexico) were inoculated hypodermically with each of two sporidial isolates of corn and *Tripsacum* smut. Twenty-one days later the number of galled plants was counted. The results obtained were as follows:

Smut	Number Galled		Number Stunted	
	Corn	Teosinte	Corn	Teosinte
Sweet corn	14/20	9/9	8	3
Sweet corn	14/20	9/9	8	3
Tripsacum	8/18	10/10	5	9
Tripsacum	0/19	0/10	0	0

The two corn smut isolates and one *Tripsacum* smut caused the most severe injury on both corn and teosinte; all of the plants stunted by these three isolates later shriveled and died. It is particularly significant that *Ustilago dieteliana* goes readily to corn and teosinte and forms galls which are not typical of the sporulation on *Tripsacum,* confirming Wallin (18). These data suggest that the *Tripsacum* smut may be only a strain of the corn smut organism and that the species *Ustilago dieteliana* is not a dis-

tinct species. The susceptibility of teosinte to the corn smut organism may be incident to its frequent hybridization with corn in the Chalco, Mexico area.

LEAF DISEASES

Helminthosporium leaf blight. Helminthosporium leaf blight is the most serious foliage disease of corn in Central America. It is caused by *Helminthosporium turcicum* Pass., a fungus that brings about rapid necrosis of the leaf tissue. Its prevalence and destructiveness in Guatemala are sharply influenced by climate and environmental conditions, as first reported in 1947 (6). In Guatemala *Helminthosporium* is destructive chiefly in the highlands, 3000 to 6000 feet altitude, where a semi-temperate high rainfall climate prevails. It occurs sparingly in the low coastal climates and the same is true at elevations above 7000 feet on the mountain slopes. The high temperatures of the coastal regions and the low temperatures of the mountain slopes are unfavorable for the development of the pathogen.

Helminthosporium was found to be most destructive where the rainfall was high, as at Coban, (7), (8), where the annual rainfall is about 120 inches. But in regions where the rainfall is less than 23 inches, the blight organism failed to become destructive. The early crop grown in the highlands and harvested in September often shows a loss of from five to, in some cases, 50 per cent. Some conception of the destructiveness can be appreciated from the fact that many U. S. single crosses are defoliated before the ears reach the milk stage. The same is true of some Guatemalan varieties, especially the early ones when grown in the highlands. In fact, the disease is of such long standing and occurs so regularly that the indigenous people, and they are the corn growers, do not consider the dying of the foliage as due to disease, but rather as the beginning of maturity. Sometimes, in such cases, the leaves are stripped and used for forage, but such diseased leaves make poor forage.

The destructiveness of the leaf blight organism in the highlands may well be responsible for the general practice of using the giganteum varieties rather than the early. The latter are more susceptible and yield less than the former. Susceptible early varieties are grown only to a limited extent when the supply of corn is insufficient to last the family until the late crop matures. The yield of the early varieties is distressingly low, often only three to eight bushels per acre. There is reason to believe that the ravages of *Helminthosporium* leaf blight and the low yielding capacity of the early varieties have been a contributing factor in the selection and use, by the indigenous people, of the late giganteum varieties in the high rainfall regions of the highlands of Guatemala.

By choosing the planting date in some localities in the highlands, one can be assured of favorable conditions for the pathogen to produce an epiphytotic, thereby providing sufficient disease for testing inbred lines and hybrids.

Seven varieties were found to be resistant, namely, 134A-46, 31-44,

159-44, 1483-45, 49A-46, 92A-46, and 20-47. These all belonged to the giganteum group. U. S. inbreds and single crosses were more susceptible than most Guatemalan corns, and top crosses of Guatemalan corns on U. S. inbreds were also more susceptible.

Corn rust caused by Puccinia sorghi Schw. Corn rust is generally distributed in Guatemala. It occurs all year around on growing corn in the uredo- and teleutospore stages (Fig. 5). The aecidial stage has never been reported from Guatemala. The mild climate and constant presence of growing corn favor the survival of the parasite without the utilization of an alternate host. In Antigua, the rust is as prevalent in the dry season on volunteer corn as it is on the regular crop planted in March and April. The lower leaves are often killed before the plants become a foot tall. This rust has not been observed to cause severe damage to corn during

FIG. 5. Corn rust. This rust causes necrosis of the leaf tissues. The plant pathogen fruits on the surfaces of the living and dead tissues.

the past six years, except in 1949, when it was prevalent and destructive in the highlands at altitudes ranging from 4000 to 6000 feet. In Antigua, the rust appeared on our plots in April and increased rapidly, although no rain fell until May 28, the beginning of the rainy season. Apparently the rust pathogen was favored by the heavy dews which often occur in the Antigua region. In a plot planted to nine U. S. single crosses and 24 U. S. inbreds intended for crossing purposes, the rust became so severe that the lower leaves of the plants were killed. The prevalence of the rust was so great that it became necessary to spray the plants with Bordeaux mixture to keep them alive. Seven of the nine single crosses, even after they had been sprayed with the Bordeaux mixture, showed a four-type injury on a scale of 0 to 5, where 0 indicated no rust and 5 necrosis of all the foliage. The 24 inbreds were injured more than the single crosses.

In an adjoining plot consisting of 38 Guatemalan inbred lines which

had been selfed twice, the rust became prevalent, though not to the same extent as on the U. S. corns. There was a wider range of injury on the Guatemalan inbreds than on the U. S. corns. Two lines, 47-44 2s and 1613-45 2s, were nearly free from rust, while the other 36 lines showed a range of 1 to 5 type of injury.

In another plot of 28 lines, inbred once, there were three lines that showed only a trace of rust present. These three lines were 192-44, 35y, and 1478-45. The other 125 lines showed a 1 to 5 type of injury. Furthermore, these three rust resistant lines occurred in two other plots where they showed the same rust reaction, only a trace of injury. The lines which showed a 3 to 4 type of reaction were very definitely injured. The Guatemalan corns as a whole showed less injury than the U. S. single crosses and inbred lines. These five lines mentioned above all belonged to the giganteum group. Eight inches of rain fell in June, which doubtless favored the rust pathogen. Teleutospores failed to form in most of the sori; instead, *Darluca filum* appeared generally in the sori.

Darluca filum parasitized the rust pathogen. The Darluca pycnidia were gregarious, globose, erumpent and brown to black. The pycnidiospores were suboblong-fusoid, straight, and finally became one septate. The dead tissues about the uredosori became black. In this blackened area an ascomycete appeared. The perithecia were submerged with ostioles extending to the surface, 142.6-173.2 $\times$ 146.2-158.5 μ; asci 57-72 $\times$ 6-9 μ, containing eight three to four celled spores per ascus; ascospores 11-19 $\times$ 3-6 μ.

The relation of the ascomycete to the Darluca has not been established culturally; however, the association of the ascomycete in the blackened tissues about the sori parasitized by the Darluca suggests a relationship.

The teleutospore stage was inhibited in its development to such an extent that it was not easy to find teleutospore sori containing spores, suggesting that the rust was destroyed by the Darluca.

Corn planted in November, 1949, and grown during the dry season was infected by rust throughout its growth period but no Darluca appeared. The same was true of the plants in a plot planted February 15, 1950, although the plants became infected when only six inches high. The rust continued to maintain itself but no Darluca appeared.

The situation was different on a plot of eight varieties planted on March 16, 1950. The rust pathogen became prevalent and destructive on these corns and Darluca appeared about June 20, a month after the rainy season began. It seems significant that the rust which developed during the dry season, November, 1949, to May, 1950, was not parasitized.

Guatemalan corn rust caused by Angiospora zeae Mains. Guatemalan corn rust is little known and was first reported in Guatemala by Mains (3). Its complete life history is not known; only the uredospore and teleutospore stages have as yet been found. This rust occurs on corn grown on the low coastal plain as well as in the highlands, but it has not been seen above 7000 feet altitude. It seldom becomes sufficiently preva-

lent to cause much damage to the foliage. The pathogen kills the tissues only in localized spots; seldom are extensive areas of leaf tissue killed. Muller (11) reports having observed the rust for seven years in Guatemala and that only once during that time did he find the parasite causing severe damage — on corn grown on the coast in 1947. However, the rust generally is more prevalent in the highlands than in the lowlands. It has been observed on corn only after flowering, never on seedlings. The large late giganteum corns seem to be more susceptible than the corns belonging to the other three groups. The corns, 3A-46, 15A-46, 16A-46, 17A-46, 19A-46, 41A-46, and 42A-46, were observed to be more heavily infected with this parasite than 33 other corns grown in the same plot at Chocola in 1946. In 1951 this parasite was observed to be most prevalent near Coban, in the Department of Alta Verapaz, and in southeast Guatemala, between Guatemala City and the El Salvador border.

Corn rust caused by Puccinia polysora Underw. The third rust found on corn in Guatemala is *Puccinia polysora*. This parasite occasionally assumes epiphytotic proportions; such an outbreak occurred in Guatemala during the summer of 1951.

The fungus was first described in 1897 on *Tripsacum dactyloides* at Auburn, Alabama, and Cummins (1) reported it on corn in Guatemala. Only the uredo and teleutospore stages have been found. The sori occur on both sides of the leaves, are irregularly scattered, and remain covered by the epidermis for some time, eventually rupturing by a longitudinal slit. The uredospores on our material are pale rusty brown, slightly echinulate, broadly oval, 26-30 $\times$ 28-34 μ. The teleutospores are irregularly angled, slightly constricted at the septum, only slightly thickened at the apex, 17-24 $\times$ 29-39 μ.

The inbred lines and varieties of corn grown at Antigua in 1951 varied in their rust reaction from a 1 to 4. It is conceivable that this pathogen could cause some loss in the highlands of Guatemala on susceptible varieties.

STEWART'S DISEASE

Stewart's disease, caused by *Bacterium stewartii,* has not been observed, although Neiderhauser (12) reports it from Mexico. Its absence in Guatemala may be due to the limited population of the species of Diabrotica known to be a vector. Our surveys for species of Diabrotica show that there are at least 12 species that appear on corn, but *Diabrotica duodecimpunctata* Oliv. has never been taken on our plots at Antigua, Coban, or Tiquisate. It definitely is not common. The other species that is known as a vector in the United States, *Acalynma trivittata* (Mann.), formerly known as *Diabrotica vittata,* is common. Another contributing factor to the apparent absence of Stewart's disease may be the absence of sweet corn. On the other hand, it is possible that the absence of Stewart's disease may be due to other factors and that further exploration will show that it does occur at times.

CORN STUNT DISEASE

At least five virus diseases have been described in corn; all of them occur in the tropical or subtropical climates. Stunt occurred in the Rio Grande valley of Texas in 1946. The author found it on sweet corn and brought it to the attention of George Alstadt, then Extension pathologist. It was recorded and described in Texas in 1946 (13). The same year, this disease was found occurring on our experimental plots in Guatemala. Kunkel (2) showed that the vector of this virus was a leaf hopper, *Dalbulus maydis*. Maramorosch (4) has obtained transmission of the virus by injecting hoppers with minute amounts of diluted plant juices pressed from diseased corn plants. The stunt disease is distinct from the other viruses in that it has a different vector and does not manifest itself until the corn is about to flower. At this time the foliage becomes light green and the plant becomes distorted. The internodes are shortened, the tassel is distorted, and an excessive development of ear shoots occurs, none of which develop normally. The plant is often definitely stunted. In other cases there may be no symptoms expressed until the plant has blossomed and the ear shoots have formed. In such cases the latent auxillary buds become active and a branch develops at each node below the ear, except on the lower portion of the stalk (Fig. 6). These lateral branches vary from a foot to four feet in length. In most cases the foliage is yellow and the floral organs are distorted. In rare cases the virus seems to stimulate the lengthening of the internodes, so that the diseased plant towers from one to three feet above the healthy ones. Apparently the virus reaches the growing point of the plant and becomes systemic in all its buds, thus affecting both the vegetative and reproductive stages of the plant. Little or no grain develops on the ears of infected plants.

The stunt disease occurs from sea level to 6000 feet, but it is more prevalent in the lowlands than in the highlands, varying from a trace to five per cent. The disease has never been observed on corn grown at 7000 feet and above.

Stunt can be confused with maize maggot injury of corn, since some of the symptoms are identical. Maize maggot injury can be distinguished from the stunt disease, in that the growing point of the plant shows evidence of the feeding of the maggot on the meristematic tissues. Stunt-diseased corn can also be confused with teosinte, due to the excessive branching already described. It can be distinguished from teosinte by the ear shoot characters. It is not improbable that some of the reports of teosinte and teosinte-corn hybrids in cornfields in Mexico and Central America were corn plants showing stunt disease symptoms.

CORN STARVATION

The chief disease of corn in Guatemala is starvation, incident to impoverished soils (low in nitrogen) and inadequate soil tillage. The depletion of the soil is caused by erosion and continuous cropping of corn. Erosion is rapid because much of the corn is grown on steep mountain sides. The fact that the rainfall is seasonal, with a wet and dry season, also

facilitates erosion because all the rain falls within a six month period, during the active growing season of the crop. Starvation is further aided by the practice of removing all plant refuse from the field before the new crop is planted, leaving little or no organic material to retard erosion or to improve the fertility of the soil. The Indian farmer attempts to over-

FIG. 6. The stunt disease causes excessive branching of the corn plant. Such plants bear no ears.

come depletion of the soil in some parts of Guatemala by resting the land, when it ceases to be sufficiently productive. Sooner or later the land becomes populated with the plants native to the region, but such a system fails to maintain the fertility, which is apparent from the low yields obtained.

The most conspicuous symptoms of starvation are: yellowing of the foliage; short, spindly stalks; sterility; small ears; poor pollination; red and white striping of the leaves; bronzing; and early firing of the lower leaves. Not all of these symptoms may occur in the same crop, and the prevalence may vary from one season to the other. Nitrogen starvation, indicated by the yellow color of the foliage, is most apparent just before the plants come into flower. The symptoms of starvation are aggravated when the crop suffers from either too much or too little moisture. The losses sustained from this cause may exceed 50 per cent.

ROOT NECROSIS

Root necrosis, caused by *Pythium* spp., may become destructive in the corn belt of the United States. In Guatemala, however, the roots of corn are mostly free of lesions caused by soil-inhabiting pathogens such as are found in the western part of the corn belt of the United States. Corn roots have been examined in the highlands and lowlands in Guatemala over a period of four years. Little root necrosis has been observed, except when the conditions for growth were unfavorable, incident to excessive rain or depletion of soil fertility. This condition is true not only for Guatemalan corns, but also for United States inbreds, single crosses, open-pollinated varieties, and hybrids, when grown in Guatemala.

Since root necrosis seems to be of little importance under present cultural practices, an attempt was made to create an environment conducive to the development of the soil-inhabiting pathogens known to cause root necrosis. A layer of dead stalks was spread over a plot of ground 10 x 15 feet after planting. The seed was sown one-half inch apart in two five-foot rows three feet apart. These rows comprised three United States hybrids and two Guatemalan varieties. Thirty days after planting, the plants were dug and examined to determine the degree of root necrosis. Less than two per cent of the plants exhibited any root lesions. The rest were strikingly free of lesions. Soil organisms may be associated with root injury such as caused by the larval stage of species of Diabrotica.

Observational and experimental data suggest that the soil environment is unfavorable for the pathogens causing root necrosis.

EAR ROTS

Ear rots are common, destructive, and more prevalent on the corns maturing in the wet season than those ripening in the early part of the dry season. It is probably safe to say that ear rots destroy or impair the quality of 2 to 12 per cent of the annual crop. The ear rots cause more

loss to the corn crop than all other plant pathogens combined. It is not uncommon to see some corn having 2 to 50 per cent discolored or moldy kernels offered for sale in the markets. In addition, such livestock as is possessed by the Indians consumes much damaged grain that never reaches the market. It is not easy to learn definitely how much ear rot develops in the field because the diseased ears are often promptly discarded. Further, the Indian farmers are not sympathetic to removal of the husks in the field so as to permit an examination of the ears.

Diplodia zeae (Schw.) Lev. is more prevalent than any of the other organisms causing ear rot (Fig. 7). At San Martin, in the Department of Huehuetenango, in late September, 1944, the crop from a two-acre field was made available for examination immediately after harvest. Eleven per cent of the ears were infected to some degree. Some of the ears were

FIG. 7. Dry rot caused by *Diplodia zeae.*

entirely overrun by the parasite, while others were only partially destroyed. *Gibberella saubinetii* (Schw.) Petch is rare. *Nigrospora oryzae* (Berk and Br.) Petch ear rot is sometimes prevalent and destructive, as in 1947. In one field near Guatemala, where a count was made at harvest time, 72 per cent of the ears in ten quintals (1000 pounds) were attacked by *Nigrospora*, and most of them were discolored and chaffy. Such serious damage has not been observed since, although it is not uncommon to find five per cent of the ears attacked. Species of *Penicillium, Aspergillus, Rhizopus, Fasarium,* etc., probably are more common and destructive than *Diplodia* after the crop has been harvested. The saprophytic fungal flora are favored by the difficulties encountered in drying the corn rapidly during the rainy season. Partial drying is accomplished in the field by the practice of doubling. Doubling consists of breaking the stalk over by hitting it with the back of the machete, just below the ear. This allows the ear to hang down, in which position there is less opportunity for moisture, spores, and insects to collect inside the husks. After doubling,

the crop is often allowed to stand in the field for as much as two months before it is gathered. This is particularly true of the late crop, but not so general a practice on the early crop. Doubling is also useful in preventing the birds from feeding on the tips of the ears. Parakeets are especially abundant and are sometimes devastating.

There are no special storage facilities for most of the corn grown. The Indian farmer generally uses his home. Some corn is hung up, but most of the crop is piled or ricked on the ground, or on pieces of boards or wood, in one corner of the one-room house. Smoking the corn by piling it on an improvised attic floor, above the open fire used for cooking, is often used to safeguard the seed. This smoke treatment is believed by the natives to prevent weevil injury. Sometimes the corn is stored in temporary grass-roofed small houses in the highland milpas.

LITTLE KNOWN DISEASES

There are several pathogens that cause minor foliage diseases of corn in Guatemala. *Physoderma zeae-maydis* Shaw causes dark brown spots on the leaf blades and sheaths. It has been observed only in the lowlands, where it has never been known to cause any damage. In the highlands one often sees small circular necrotic spots on the leaves, on the surface of which occurs *Cercospora zeae* Maubl. *Helminthosporium carbonum* Ulls. and *Phyllacora maydis* Maubl. may be found but cause little injury.

The destructive diseases caused by *Sclerospora maydis* Palm and *S. philippinensis* Western, common in the old world tropics, have never been observed, although climatic conditions comparable to those in Indonesia and the Philippines exist in the lowlands of Guatemala and in Central America. Varieties grown in Guatemala, when grown in Indonesia, proved susceptible in field plot studies in 1952. The downy mildew problem of corn should be studied from the standpoint of further safeguarding against the entrance of these tropical species of Sclerospora into the American tropics. *S. graminocola* (Sacc.) Schroet., found on corn in Iowa (5), and *S. macrospora* Sacc., recently described by Ullstrup (17), have never been observed in Guatemala.

SUMMARY

Little is known about the reaction of the different pathogens to the diversified climates and the existing strains of corn. However, certain tentative statements may be made, based on the observations and data in hand. Some plant pathogens are quite cosmopolitan, occurring in a wide range of climates; others are restricted. Helminthosporium leaf blight is not prevalent in low tropical or mountainous climates. The disease is common in the highlands (2000 to 6000 feet altitude) where the rainfall ranges from 40 to 60 inches. The ear rot organisms are less destructive in the semi-arid climates than in the wet regions.

Seedling and root diseases are little known. Ear rots in the field and in the process of curing take a heavy toll of the crop. The losses often

range from 2 to 12 per cent. Leaf diseases show a greater variability in injury than the ear rots. The losses probably do not equal those caused by the ear rots. *Helminthosporium* leaf blight and corn rust are the most serious leaf diseases.

Corn smut is not common, but head smut may, in some fields, cause a 1 to 12 per cent loss. The smut that occurred on *Tripsacum latifolium* was readily transferred to corn and teosinte, suggesting that *Ustilago dieteliana* may not be a valid species.

The only disease control measure practiced consists of doubling the stalk just below the ear when the grain is nearly mature, but not low enough in moisture content to harvest. This practice permits rapid drying of the ear, which inhibits the further growth of ear rot organisms.

Most of the corn diseases that are present in Guatemala also occur in the United States, which indicates that most pathogens followed corn when it was moved from its place of origin to the north temperate zone.

Stewart's disease and two of the downy mildew diseases occur in the United States, but have not been found in Guatemala.

There are differences in the resistance of corn varieties and strains to *Helminthosporium turcicum* and *Puccinia sorghi.* As has been cited, seven varieties are resistant to the leaf blight organism and five to the rust organism. They all belong to the giganteum group of varieties.

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CYTOLOGICAL AND EMBRYOLOGICAL BASIS FOR STERILITY IN AUTOTETRAPLOID SWEETCLOVER, MELILOTUS ALBA DESR.[1]

BIDHAN CHANDRA DAS[2]

Departments of Botany and Agronomy
Iowa Agricultural Experiment Station

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The production of mature viable seed is the climax of a series of morphological and cytological processes that are subject to genetic and physiological control. Aberrations of one or more critical events or processes during the reproductive cycle may result in varying degrees of reduction in the yield of viable seed.

Many artificially produced autotetraploids are characterized by low fertility. This undesirable feature raises questions of theoretical and practical importance. A study of meiotic behavior, fertilization, and embryology may reveal correlations between fertility level and specific aberrations in the reproductive process. Such information is particularly valuable for a comparison of the consequences of natural selection in naturally occurring autotetraploids, with the effects of experimental selection for fertility level in artificially produced autotetraploids.

The cytology of the male gametophyte of autotetraploids has been studied more extensively than that of the female gametophyte. The assumption that the cytology of the female gametophyte is essentially the same as that of the male gametophyte needs verification, especially in species that exhibit high sterility.

The embryological basis of seed failure is know in a number of plant species, and failure is known to be due to different causes in different species. Failure of seed development may take place at various stages of embryonic growth. Therefore, it is desirable to investigate the specific morphological aberrations that influence seed development in a given species.

Autotetraploid sweetclover, *Melilotus alba*, developed by colchicine treatment (16), was found to be generally low in fertility, but rather

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wide differences were found among individual plants and inbred lines (5). The present study was undertaken to determine the basis for different levels of fertility in some of the available lines of autotetraploid sweetclover, particularly with respect to the cytology of the megasporocyte, embryo sac development and subsequent embryonic growth.

REVIEW OF PERTINENT LITERATURE

Reduction in fertility has been attributed to morphological and histological abnormalities during seed development, cytological irregularities, physiological conditions of the mother plant, presence of self-sterility genes, and various other factors. Only the more pertinent papers will be reviewed here.

Brink and Cooper (2) and Brink, Cooper, and Albrecht (8) reported that the low fertility in self-pollinated *Medicago sativa* is due only in part to restricted pollen tube growth. The most significant factor in the reduction of fertility following selfing in *Medicago* is the collapse of fertile ovules during development. Abortion of fertile ovules takes place at various stages in development, but usually in the proembryo or young embryo. Brink, Cooper, and Albrecht (8) also observed that the first signs of disintegration of unfertilized ovules appear 48 hours after pollination. Cooper (6) described megasporogenesis in diploid *Melilotus*.

Brink and Cooper (3) reported that abortion of fertile ovules in *Medicago* takes place through the aggressive growth of the inner integument. This type of failure of seed development, known as somatoplastic sterility, is associated with inability of the endosperm to keep pace with this overgrowth. The authors also observed that up to six days after pollination the number of cells in the embryo increases in arithmetic progression and that of the endosperm increases exponentially. The growth of the endosperm is significantly lower after selfing than after hybridization.

Satina, Rappaport, and Blakeslee (39) reported the formation of ovular tumors in fertilized ovules of *Datura*. This formation of tumors prevents successful development of seed after hybridization between incompatible species crosses. Tumor formation results from hyperplasia of the endothelium. Tumor growth becomes retarded as the endosperm and embryo become absorbed progressively, and tumor growth stops after the endosperm and the embryo are completely absorbed.

Dorsey (10) reported that chromosome doubling is the basis for sterility, or very low fertility in *Triticum monococcum, Avena brevis*, and *Hordeum distichon*. In *H. vulgare*, six-rowed barley, all tetraploids produced spikes with seeds, but mature seed set was very low.

Reduced fertility in induced autotetraploids has been attributed by Darlington and Kostoff (9, 19) to meiotic irregularities. According to Kostoff (19, 20), chromosome size is an important factor in determining the fertility level of polyploids. Autotetraploids with small chromosomes have fewer chiasmata, form fewer multivalents, and for this reason are more fertile.

Raptopoulos (37) reported an inverse relationship between fertility and the mean number of quadrivalents in autotetraploid *Prunus*. Similarly, Belling and Blakeslee (1) reported the formation of 12 quadrivalents in tetraploid *Datura*. Non-disjunction in diploids was rare but in tetrapoids 25 per cent non-disjunction was found in microsporocytes.

Lesley and Lesley (22) found 7 to 12 quadrivalents at diakinesis in microsporocytes of a tetraploid *Lycopersicum*, which was derived from a cross between a double trisomic and a diploid. This tetraploid produced seed sparingly when selfed. Counts of second metaphase plates indicated unequal chromosome distribution.

According to Matsuda (25) chromosomes of tetraploid *Petunia* form tetravalents as well as multivalents. Non-disjunction of chromosomes occur at anaphase I, and lagging chromosomes were observed in both divisions.

Tanaka (40) reported that in tetraploid *Carex*, 30 per cent of the gametes were unbalanced, due to meiotic irregularities. The observed proportion of unbalanced gametes accounted for reduction in fertility. Tanaka also indicated that meiotic irregularities in the tetraploid were due chiefly to irregular separation of the metaphase I multivalents. Similarly, Upcott (41) found that in tetraploid *Lycopersicum*, non-disjunction resulted in the formation of more than 30 per cent unbalanced gametes. It was also observed that the occurrence of non-disjunction was almost entirely dependent upon the formation of quadrivalents.

The occurrence of trivalents, tetravalents, and multivalents was found in tetraploid *Pyrus* by Vaarama (42). Lagging univalents also were observed in anaphase I. Elimination of univalents took place frequently. Lagging chromosomes also were common in anaphase II.

Myers (29) studied meiosis in autotetraploid *Lolium perenne* and observed irregular orientation on the plate of metaphase I. Frequently, members of quadrivalents disjoined unequally at anaphase I. Lagging univalents, which resulted in some cases from incomplete disjunction of quadrivalents, also were found at anaphase I. The frequency of univalents at metaphase I was high, and these univalents tended to lag and divide equationally at anaphase I and to remain in the cytoplasm at telophase I and II. The frequency of metaphase I univalents in this artificially induced autotetraploid was higher than in naturally occurring autotetraploid grasses, such as *Dactylis glomerata*.

Relationships between chromosome behavior and seed setting have been found in *Dactylis glomerata*, *Arrhenatherum elatius* and *Agropyron cristatum* by Myers (28) and Myers and Hill (30, 31, 32). Significant negative correlations were found between seed set and increased percentages of univalents, laggards, and quartets with micronuclei. Significant differences in chromosome behavior during meiosis were found among different plants within a species, and between plants of the first inbred generation of *Dactylis glomerata*. This indicated heritable differences in meiotic behavior among lines. The authors suggested the possibility of selection for increased fertility in these autotetraploid grasses.

Cooper (7) reported lagging chromosomes in both divisions of meiosis in autotetraploid *Medicago* (2n = 64). Chromosome behavior in macrosporogenesis was similar to that of microsporogenesis.

Meiosis in autotetraploid *Secale* was studied by O'Mara (33), who described multivalent association, inversion or duplication bridges, univalent bridges, univalent laggards and non-laggards, irregular anaphase distribution, and chromosome loss.

Jorgensen (17) found that meiosis was very regular in tetraploid *Solanum* and the chromosomes were associated as bivalents in most cases. No correlation between reduced fertility and meiotic irregularities or number of quadrivalents was found by Lindstrom and Humphrey (23), Lindstrom and Koos (24) and Humphrey (15) in autotetraploid *Lycopersicum,* and by Randolph (34, 35) in autotetraploid maize. According to Randolph (36), homozygosity *per se* is responsible for reduced vigor and fertility of the more homozygous tetraploid maize. Randolph (36) also indicated that although meiotic irregularities may account for 10 to 15 per cent reduction in fertility of tetraploids, physiological and genic disturbances associated with chromosome doubling are more important causes of sterility.

According to Fischer (13), genetic factors are more important causes of high sterility in tetraploid maize than chromosomal irregularities during meiosis. Müntzing (26), assumed that the degree of fertility in experimental autotetraploids is not necessarily correlated with the frequency and behavior of quadrivalents, and that physiological conditions of the mother plant are a more important factor than the constitution of the spores. Aneuploidy was regarded to be an important factor in causing a high degree of sterility in tetraploid *Secale* by Müntzing (27) and in tetraploid *Lactuca* by Einset (12).

High sterility of autotetraploid *Secale,* as compared with the diploid, was found to be due to increased failure of fertilization and increased frequency of slow and interrupted development of endosperm in the tetraploid (14). Einset reported that the chief causes of sterility in *Lactuca* were ovule abortion, due to meiotic irregularities during megasporogenesis, and to failure of pollen germination and pollen tube growth (11). Gametophyte abortion was found to account for 65 to 85 per cent of the sterility in *Hilaria* (4).

Chen (5) studied the inheritance of self-fertility in autotetraploid *Melilotus alba* and found that average self-fertility of the progenies was in good agreement with the parents from which they were derived. A highly significant parent-progeny correlation of 0.675 was found. In the same stocks, Johnson and Sass (16) reported chromosome lagging and markedly irregular distribution in anaphase I. Univalent, bivalent, quadrivalent, and occasional trivalent associations were observed. However, the reduced fertility of this autotetraploid cannot be explained entirely on the basis of pollen condition.

From a study of interspecific hybrids of *Melilotus,* Webster (43) showed a positive correlation between the percentage of stainable pollen

and self-fertility in the F_1 plants. Ovule abortion also was noted. It was concluded that pollen abortion is conditioned by factors other than meiotic irregularities. The author suggested a hypothesis of pollen degeneration resulting from certain lethal gamete combinations.

MATERIALS AND METHODS

The lines of autotetraploid *Melilotus alba* used in this study were developed in the sweetclover improvement program of the Iowa Agricultural Experiment Station. Four lines were used, two relatively high in self-fertility and two of low self-fertility. Seeds from self-pollination of these lines were planted in four-inch clay pots in the greenhouse. Cuttings from each line were made and rooted in vermiculite and later transplanted to four-inch clay pots in a sterilized mixture of soil, peat, and sand. Each pot was given nutrient solution as needed. Early in October, the photoperiod was extended to 18 hours by the use of 200 watt Mazda lamps. With this treatment flowering was initiated early in November.

As soon as the plants came into bloom, self-fertility tests were made within each line. Flowers were tripped with a toothpick as they progressively opened along a raceme for a period of two to three days. All plants were self-pollinated during the same period to eliminate possible confounding of fertility level with environmental changes.

The highest-fertility plants in the high-fertility lines, and the lowest-fertility plants in the low series were selected for detailed study. Selection of plants within lines was necessary because the high-fertility lines were not homozygous for fertility levels.

The four lines used in the present study, with their respective fertility levels in 1950, are shown in Table 1. The rating of self-fertility was based on per cent seed set, after hand-pollination under controlled greenhouse condition.

The period of collection of material for pre-fertilization studies, extended from July, 1951, to March, 1952. Meiotic stages were most abundant in young flower buds in which pollen was just changing color from green to yellow, a change which was easily detected with a magnifying glass. The most numerous figures were obtained from the materials collected between 9:30 A.M. and 10:30 A.M. in full sunlight, in January and February.

Pollination for subsequent collection of material for post-fertilization and embryological studies was made during the period from January to March, 1951. Freshly opened flowers were prepared for pollination by removing the petals with forceps. Flowers were then tripped with a toothpick to scarify the stigmatic surface and to transfer pollen from the freshly opened anthers. Pollinations in the two high-fertility lines were made concurrently, and the same procedure was followed with respect to the two low-fertility lines. Collections of pistils were made from 0 hour to 12 days after pollination. The intermediate collections were at 12, 18, 24, and 36 hours and two, three, four, six, eight, and ten days after pollination.

A Nawaschin type formula, Craf III, was used for killing floral buds and pistils, and a dioxan-normal butyl alcohol series was used for dehydration (38). Materials were embedded in paraffin. Proper orientation in the paraffin block was necessary to permit sectioning in the correct plane. For the pre-fertilization study, four to five floral buds were put side by side in the block in order to section the group in one operation, and three to four ribbons totaling 12 to 20 flowers were mounted on a slide. Sections were cut eight to ten microns in thickness and stained with iron-hematoxylin (38).

OBSERVATIONS

The megasporocyte is of hypodermal derivation, and attains considerable size before the integument primordia have become well defined (Fig. 1). The sporocyte seems to be normal during prophase I.

TABLE 1

Fertility Levels of Four Lines of Sweetclover

Lines	Per cent Self-fertility
High lines:	
11–1	36.0
8–2	20.2
Low lines:	
7–5	10.8
2–5	2.6

Early prophase cannot be analyzed profitably because of the large number of very small chromosomes. In late prophase the chromosomes become shortened and thickened and typical diakinesis figures are evident (Fig. 2). In these figures, bivalent, quadrivalent, and occasional univalent and trivalent associations have been observed.

Chromosome counts can be made in metaphase plates, but chromosome associations can be determined only in favorable preparations. In normal metaphase figures, all the chromosomes are oriented in a regular manner on the plate (Fig. 3). More commonly, metaphase figures exhibit various abnormalities, in which the chromosomes are scattered irregularly toward the poles, or lag outside the spindle (Figs. 4, 5, 6, 8). Univalents occur frequently at metaphase I (Figs. 4, 5). In some cells chromosomes are clumped into deeply stained masses (Figs. 4, 7).

Normal anaphase separation is comparatively rare, especially in low-fertility lines (Figs. 9, 17). The presence of lagging chromosomes characterizes most of the anaphase figures examined in this study. The extent of lagging varies greatly. In some cells one or two laggards are present (Figs. 10, 13, 89), whereas in other cells three or more laggards occur (Figs. 11, 12, 14, 16, 18, 19). Some of the laggards may be near the polar groups (Figs. 11, 14, 19), or they may be outside the spindle

(Figs. 15, 18). In most cases these laggards are univalent or possibly dividing univalent chromosomes (Figs. 10, 14, 18, 19), whereas some lagging chromosomes seem to be bivalent on the basis of mass, when compared with the frequent univalents (Figs. 16, 18, 19).

In addition to frequent lagging in anaphase I, other chromosome aberrations have been observed. In low-fertility lines, irregular scattering of the chromosomes of one of the polar groups is frequent (Fig. 15). The chromosomes of one polar group of a cell may be spread out along the spindle and in the surrounding cytoplasm, whereas the other polar group seems to be in the process of interphase reconstitution. Chromosome clumping is also common in anaphase I (Fig. 20). This condition is more pronounced in low-fertility than in high-fertility lines.

During telophase I, the spindle becomes expanded laterally, and the chromosomes appear to be smaller than in metaphase or anaphase (Figs. 23, 25). Lagging chromosomes, which appear to be univalent in most cases, are also evident in this stage (Figs. 21, 24, 90, 91). As many as six to seven laggards may be evident (Figs. 22, 23, 25). Other cells exhibit only one or two lagging chromosomes (Figs. 21, 24). In a few cases stray chromosomes occur beyond an interphase nucleus, outside the spindle (Fig. 21). This seems to be associated with the frequent presence of non-oriented chromosomes at metaphase. During late telophase well-defined interphase nuclei develop (Figs. 21, 25).

Lagging or non-orientation of chromosomes also is evident in metaphase II (Figs. 26, 27). One of the nuclei of the diad may be in metaphase II, whereas the other nucleus may still be in prophase II. Various chromosome aberrations also occur in anaphase II. Laggards occur in one of the cells (Fig. 31), or in both cells (Figs. 29, 30, 92). Some anaphase II cells have very abnormal chromosome distributions (Fig. 31). One nucleus may undergo normal separation whereas the other nucleus may exhibit considerable irregularity (Figs. 28, 31, 32, 34). An abnormal nucleus may consist of a single heavily stained mass (Fig. 28), or its chromosome complement may be in three to four compact, deeply stained groups (Figs. 32, 34). Anaphase separation in two nuclei of the diad is not always simultaneous; one of the cells may be in anaphase II and the other in metaphase II (Fig. 33) or in telophase II. Irregular chromosome distribution is also evident in telophase II (Figs. 35, 36).

Irregular chromosome distribution is of more frequent occurrence in low-fertility than in high-fertility lines. The low-fertility lines commonly exhibit extreme meiotic irregularity (Figs. 7, 15, 18, 19, 20), which seems to be associated with subsequent embryo sac abortion. Scattering of chromosomes of one polar group during anaphase I and telophase I was also observed in the two low-fertility lines studied (Fig. 15). Abnormality in metaphase, primarily the presence of univalents and non-oriented bivalents, seems to be of common occurrence in both low- and high-fertility lines (Figs. 4, 5, 6, 8). Clumping of chromosomes was more frequent in the low-fertility lines (Figs. 7, 18, 19, 20, 32, 34), than in high-fertility lines (Figs. 4, 28).

FIG.

1. A young ovule of autotetraploid *Melilotus alba,* showing megasporocyte and short integuments. 170×.

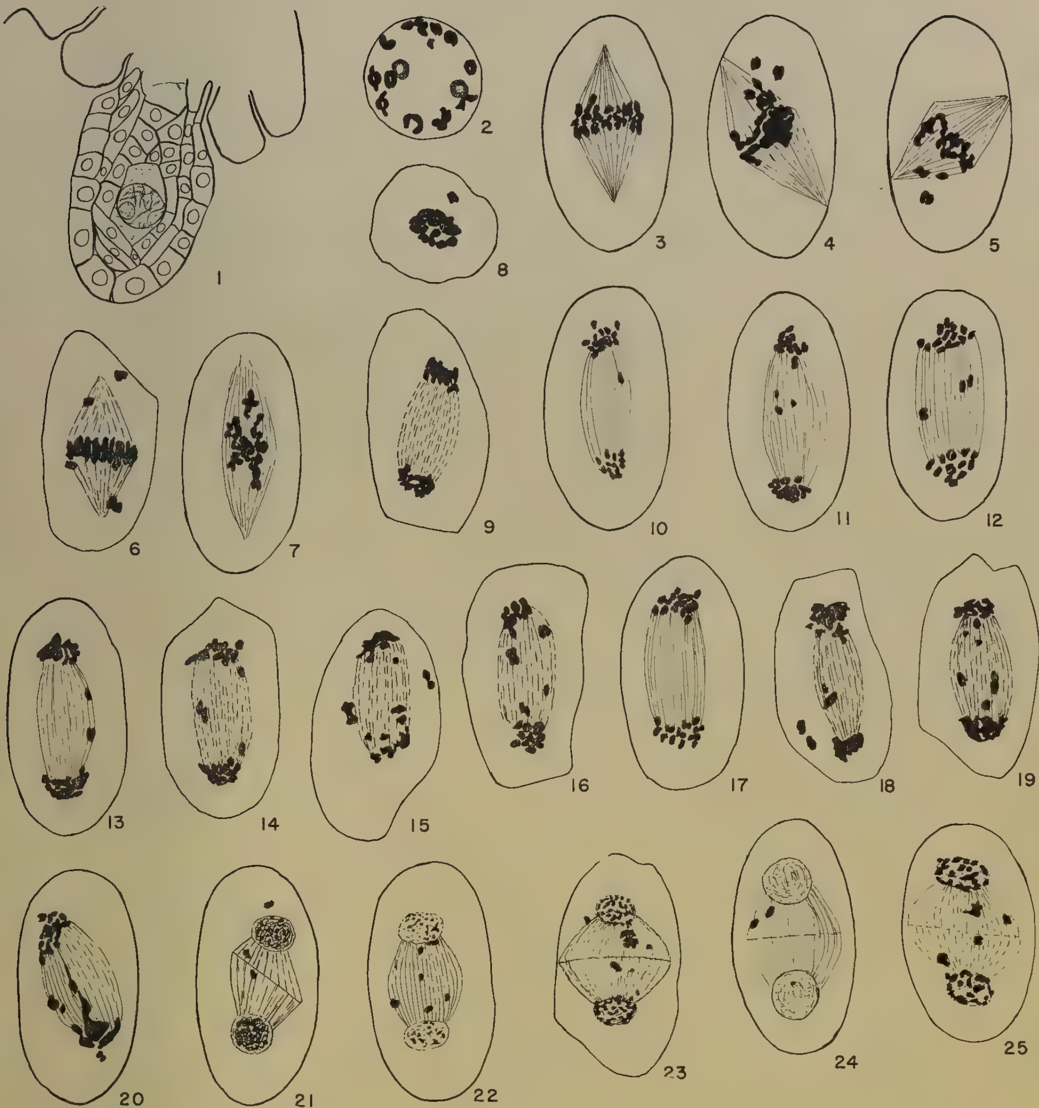
Meiosis in megasporocytes. 430×. Figs. 2–25.

2. Megasporocyte at diakinesis.
3. Normal metaphase I, highest-fertility line.
4. Abnormal metaphase I, with some chromosomes outside the spindle, highest-fertility line.
5. Abnormal metaphase I, showing univalent, also non-oriented bivalents, one of which is outside the spindle, medium-low-fertility line.
6. Abnormal metaphase I with univalents, non-oriented bivalents, and lagging chromosomes outside the spindle, medium-low-fertility line.
7. Abnormal metaphase I, showing chromosome clumping, lowest-fertility line.
8. Polar view of metaphase I with bivalent chromosome outside the plate, lowest-fertility line.
9. Normal anaphase I, highest-fertility line.
10. Anaphase I, showing univalent laggard, highest-fertility line.
11. Anaphase I with lagging chromosomes, highest-fertility line.

12–13. Anaphase I, showing laggards, medium-high-fertility line.

14–15. Anaphase I with laggards near poles, medium-low-fertility line.

16. Anaphase I, showing very irregular distribution of lagging chromosomes, medium-low-fertility line.
17. Normal anaphase I, lowest-fertility line.
18. Anaphase I, showing very irregular distribution of chromosomes. Note the chromosomes outside the spindle, lowest-fertility line.
19. Abnormal anaphase I, showing laggards and clumping of chromosomes at one pole, lowest-fertility line.
20. Very irregular anaphase I separation. Note the clumping and attenuation of chromosomes, lowest-fertility line.
21. Telophase I with lagging chromosomes. Note one laggard beyond a polar group, highest-fertility line.
22. Telophase I, showing at least five laggards, highest-fertility line.
23. Telophase I with laggards, highest-fertility line.
24. Telophase I with two lagging chromosomes, medium-low-fertility line.
25. Telophase I with irregular chromosome distribution, lowest-fertility line.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A compilation of the observations of megasporocytes is given in Table 2. It is evident that irregular and abnormal separations at anaphase I and II and telophase I and II are more frequent in low-fertility lines. Only a few cells of the medium-high-fertility line were examined, because this line did not flower regularly under greenhouse conditions.

After the second meiotic division, a row of four megaspores is formed (Figs. 37, 38). The plane of the second division is frequently oblique, in which case the megaspores may not be strictly linear (Fig. 38). Four apparently normal megaspores were observed in most of the ovules examined, except in some ovules of the low-fertility lines, in which megaspore abortion occurred after meiosis. The chalazal megaspore enlarges, whereas the micropylar ones degenerate (Fig. 39). The chalazal megaspore gives rise to the 8-nucleate embryo sac.

Somatic cells of the ovule and ovary exhibit irregular chromosome behavior during mitosis. Lagging of chromosomes leads to the exclusion

TABLE 2

Results of Examination of Megasporocytes of Four Lines of Sweetclover

Lines	Dividing Cells Examined	Normal	Abnormal
Highest-fertility	91	41	50
Medium-high-fertility	11	4	7
Medium-low-fertility	98	17	81
Lowest-fertility	55	10	45
Total	255	72	183

and irregular distribution of chromosomes in the dividing somatic cells (Figs. 40, 41). In some young ovules, hypodermal cells, from which the sporocyte is derived, were found to have mitotic irregularities.

The frequency of meiotic figures exhibited diurnal periodicity. Collections were made at four-hour intervals over a 24-hour period. Collections made in the morning yielded more abundant meiotic figures than those collected in other periods. The best results were obtained from materials collected between 9:30 A.M. and 10:30 A.M., in full sunlight in January and February.

Most of the ovules in autotetraploid *Melilotus alba* develop normal embryo sacs, in both the high and low fertility lines (Fig. 54). The egg cell occupies a position lateral to the synergids. The polar nuclei lie just basal to the egg apparatus. Antipodals disintegrate early in the development of the ovule.

Deviations from normal ovule development occur in the medium-high line and in both low lines. Proliferation of the inner integument has

been observed in the micropyle (Fig. 55), and in these abnormal ovules the embryo sac has undergone disintegration.

Fertilization occurs 18 to 24 hours after pollination. The occurrence of fertilization can be detected by the presence of pollen tube debris in the micropyle, the presence of a zygotic cell wall, (Figs. 43, 44, 93), a primary endosperm nucleus (Fig. 46), endosperm nuclei (Figs. 42, 56), and by zygotic division (Fig. 43). The fertilized egg rarely divides unless several endosperm nuclei have been formed by the division of the primary endosperm nucleus. The incidence of fertilization was found to be higher in high-fertility lines than in the low-fertility lines. The first zygotic division was observed to occur 36 hours after pollination in the highest-fertility line (Fig. 43).

Two days after pollination, three- to four-celled proembryos and numerous endosperm nuclei were found in ovules of the high lines (Figs. 45, 57, 58). Two days after pollination, two- to three-celled proembryos, associated with multinucleate endosperm, may be present in the low-fertility lines (Figs. 83, 94), but this condition is less common in the low lines than in the high lines; in the low lines, most of the ovules have a zygote and an undivided primary endosperm nucleus (Fig. 70), or a disintegrated sac (Fig. 82).

Three to four days after pollination, the filiform proembryo gives rise to a 2- to 4-celled terminal globe (Figs. 47, 48, 60) in the high-fertility lines. In all these cases endosperm was found to be multinucleate. In some cases the retarded embryonic development shown in Figure 59 is associated with sparse endosperm. In the low-fertility lines few of the proembryos initiate the terminal globe (Figs. 72, 95). In some instances one or two divisions were observed to have occurred in the endosperm.

Five to six days after pollination, the embryo becomes multicellular in the high lines (Figs. 49, 61, 62, 96). In some instances the endosperm is cellular (Fig. 61). Even at this age, some ovules exhibit the undivided zygote (Fig. 74), and the undivided primary endosperm nucleus. At the same stage of growth in the medium-high-line, normal ovules have multicellular embryos (Fig. 75), and normal, free nucleate endosperm. In the lowest-fertility line embryonic growth is subnormal, in contrast with the other three lines during the same period of development (Figs. 85, 86). At this age the occurrence of a well-developed zygote and undivided primary endosperm nucleus is also frequent.

Eight days after pollination, the cotyledon primordia may be initiated, and the endosperm becomes cellular in both high and low lines. In some cases, however, in the high as well as low lines, the embryo may reach only the advanced globe stage (Figs. 50, 51, 63, 77), associated with non-cellular endosperm, or cellular endosperm (Fig. 76).

The cotyledons can attain considerable size ten to twelve days after pollination in both high and low lines (Figs. 52, 53, 69, 88). Abnormal embryos and various degrees of endosperm abnormality were noted during this advanced period of embryonic development in both high- and low-fertility lines (Figs. 64, 60–68, 78–80, 87).

Completion of meiosis in ovule of autotetraploid *Melilotus alba*. 430×. Figs. 26–36.

FIG.
26. Metaphase II with lagging chromosomes.
27. Metaphase II with laggard in one nucleus, medium-low-fertility line.
28. Anaphase II, showing normal division in the chalazal nucleus and chromosome clumping in the micropylar nucleus, highest-fertility line.
29. Anaphase II with laggards, medium-low-fertility line.
30. Anaphase II, showing laggards in both nuclei, medium-low-fertility line.
31. Anaphase II, with normal division in chalazal nucleus and abnormal chromosome separation in the micropylar nucleus, medium-low-fertility line.
32. Abnormal anaphase II. Note one laggard in the chalazal nucleus and the clumped condition in the micropylar nucleus, lowest-fertility line.
33. One nucleus is in anaphase II, with one laggard near a pole, whereas the other nucleus is in metaphase II, with a chromosome outside the spindle, lowest-fertility line.
34. Normal anaphase II in chalazal nucleus. Chromosomes of micropylar nucleus are clumped in three compact groups, lowest-fertility line.
35. Telophase II with laggards in one nucleus, highest-fertility line.
36. Telophase II showing several laggards, lowest-fertility line.
37. Linear row of four megaspores. 170×.
38. Four megaspores that are not strictly linear. 170×.
39. Ovule showing functional megaspore and three disintegrating micropylar megaspores. 170×.

40–41. Anaphase in somatic cells of ovary, showing lagging chromosomes. 1020×.


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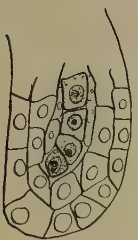
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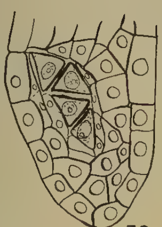
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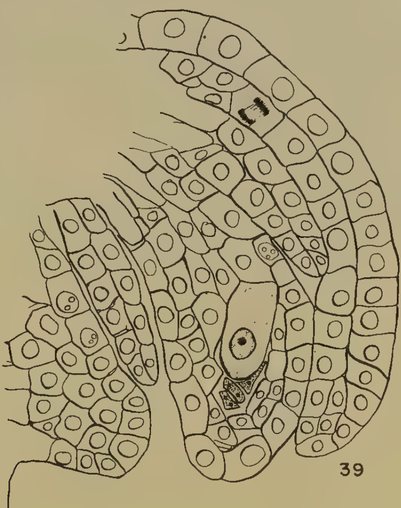
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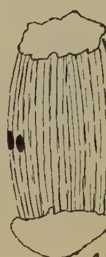
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Embryo development in the highest-fertility line 11-1. Figs. 42–53.

FIG.
42. Zygote and endosperm nuclei, 24 hours. 85×.
43. Zygote in process of division, 36 hours. 170×.
44. Zygote with primary endosperm nucleus, 36 hours. 170×.
45. Proembryo, two days. 170×.
46. Zygote with primary endosperm nucleus, two days. 170×.
47. Proembryo, four days. 170×.
48. Proembryo with several endosperm nuclei, four days. 85×.
49. Proembryo, six days. 85×.
50–51. Proembryo, eight days. 85×.
52. Embryo, ten days. 43×.
53. Embryo, twelve days. 29×.

Embryo development in the medium-high-fertility line 8-2. Figs. 54–65.

FIG.
54. Micropylar end of mature embryo sac at time of pollination. 85×.
55. Integumentary outgrowth through micropyle, associated with abnormal embryo sac, at time of pollination. 85×.
56. Zygote with endosperm nuclei, 18 hours. 85×.
57–58. Proembryo, two days. 170×.
59. Proembryo, three days. 85×.
60. Proembryo, four days. 85×.
61. Proembryo with cellular endosperm, five days. 85×.
62. Proembryo, six days. 85×.
63. Proembryo, eight days. 35×.
64. Proembryo in collapsing ovule with free-nucleate endosperm, ten days. 43×.
65. Proembryo in collapsing ovule, ten days. 43×.


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Embryos in medium-high-fertility line 8-2. Figs. 66–69.

FIG.
66–68. Abnormal proembryos, twelve days. 43×.
69. Normal embryo, twelve days. 14×.

Embryo development in the medium-low-fertility line 7-5. Figs. 70–80.

FIG.
70. Zygote and primary endosperm nucleus, three days. 170×.
71. Proembryo in collapsing ovule, four days. 170×.
72. Proembryo, four days. 85×.
73. Proembryo in collapsing ovule, five days. 170×.
74. Zygote, six days. 170×.
75. Proembryo, six days. 85×.
76. Proembryo with cellular endosperm, eight days. 43×.
77. Proembryo, eight days. 85×.
78. Proembryo in collapsing ovule, ten days. 85×.
79–80. Proembryos in collapsing ovules with free nucleate endosperm, ten days. 43×.

Embryo development in the lowest-fertility line 2-5. Figs. 81–88.

FIG.
81. Diagram of entire ovary showing the collapsed unfertilized ovules, two days after pollination. 14×.
82. Abnormal embryo sac, at the time of pollination. 85×.
83. Proembryo, two days. 170×.
84. Collapsing ovules, three days. 14×.
85. Proembryo, five days. 85×.
86. Proembryo, six days. 85×.
87. Embryo, twelve days. 43×.
88. Embryo, twelve days. 14×.


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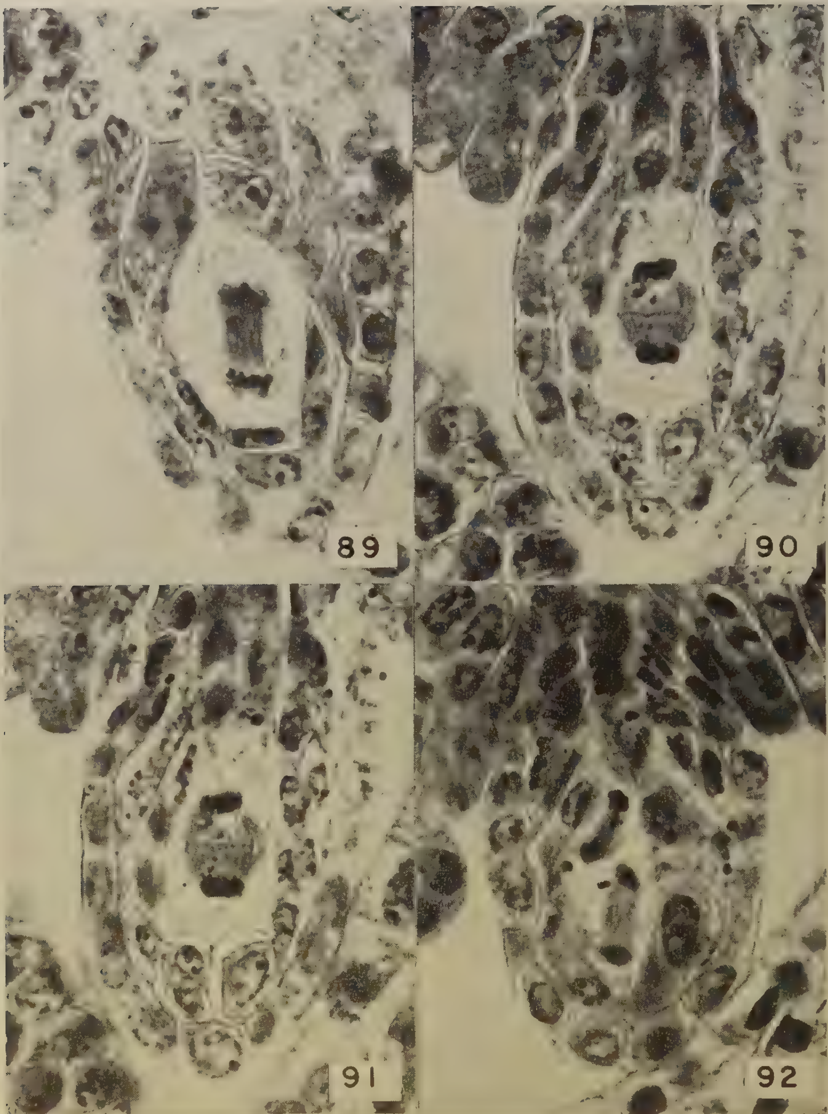

FIG.
89. Anaphase I, showing one laggard, lowest-fertility line. 960×.
90. Telophase I, showing laggards. Note the laggards near the polar groups, lowest-fertility line. 960×.
91. Same ovule as Figure 90, photographed at a different focus, showing laggards in other positions, lowest-fertility line.
92. Telophase II with laggards on both spindles, highest-fertility line. 960×.

FIG.
93. Zygote of highest-fertility line, two days after pollination. 400×.
94. Two-celled proembryo of lowest-fertility line, four days after pollination. 400×.
95. Filiform proembryo of lowest-fertility line, four days after pollination. 400×.
96. Proembryo at globe stage, medium-high-fertility line, six days after pollination. 200×.

The earliest evidence of collapse of unfertilized ovules in both high- and low-fertility lines may be found 36 hours after pollination. The collapsing ovules take a very light stain as compared with the normal ones, and the embryo sac becomes abnormal. Collapse of unfertilized ovules becomes very marked two to three days after pollination (Figs. 81, 82, 84).

The collapse of apparently fertilized ovules may take place at two ages during development. Most of these ovules collapse four to six days after pollination. During this stage of collapse, these ovules have zygotes or proembryos (Figs. 71, 73); however, the primary endosperm nucleus usually has not divided.

Collapse of well-developed ovules 8 to 10 days after pollination also occurs in both high lines and in the medium-low line. During advanced stages of development these collapsing ovules were found to have embryos of considerable size (Figs. 64, 65, 78, 79, 80). In most of these collapsing ovules, the endosperm was in the free-nucleate state (Figs. 64, 79) and in others endosperm appeared to be lacking. Collapse of nucellar and integumentary cells, which obliterate the embryo sac, was observed in some of these collapsing ovules.

DISCUSSION

The low fertility of artificially induced autotetraploids has been ascribed in some cases to genetically unbalanced or aneuploid male gametes, which are the result of irregular chromosome distribution during microsporogenesis (1, 25, 40, 41, 42). The regular meiosis reported by Lindstrom and his associates (15, 23, 24) in a highly sterile autotetraploid *Lycopersicum* was reinvestigated by Upcott (41), who found non-disjunction from quadrivalents. He assumed that some of the numerically balanced gametes were genetically unbalanced and estimated that 40 per cent of the male and female gametes were functional. Upcott's results bring the case of *Lycopersicum* into line with the other studies of microsporogenesis in autotetraploids.

Müntzing (26) discussed the above study and stated that Upcott's conclusions cannot be accepted, because the proportion of functional pollen grains would suffice to fertilize all functional ovules, and because haplontic sterility is generally less pronounced in the ovules than in the pollen. Physiological disturbances and diplontic sterility were regarded by Müntzing to be chiefly responsible for the low fertility in such cases.

As in the foregoing studies, meiotic irregularities in the microsporocyte of autotetraploid *Melilotus alba* are certainly a factor, but not the sole factor in sterility (16).

The cytology of the megasporocyte and female gametophyte of autotetraploids has not been studied extensively, probably because of the time-consuming technical difficulties of precise serial sectioning of minute ovaries. There is no comparison with the relative ease and speed with which great numbers of microsporocytes can be studied in smear preparations. It has been assumed that the cytological behavior of the female

gametophyte is essentially similar to that of the male gametophyte (26), but this may not always be true, as shown by Brown and Coe (4).

Abnormal embryo sacs in autotetraploid *Lactuca* (11) and in *Hilaria* (4) are known to be associated with abnormal meiosis during megasporogenesis, and aneuploid plants are much more sterile than euploid plants (12). The present study has shown a similar relationship between abnormal embryo sacs and sterility in autotetraploid *Melilotus alba*, and this may well be the case in the numerous autotetraploids in which the embryo sac has not been studied.

Dissimilar chromosome number in the gametes of the parents influences post-fertilization processes. In certain reciprocal crosses in *Avena* (18), if the male parent has the higher chromosome number, it gives a higher "activating stimulus," which produces rapid cell division in the embryo and endosperm, whereas, if the male has the lower number, embryo and endosperm growth is retarded. Similarly, in *Medicago* (21), differences in the rate of seed development in reciprocal crosses between 2n and 4n species has been ascribed to different rates of physiological activity and cell division in the endosperm, as well as to the dissimilar chromosome number in the parents.

The problem in autotetraploid *Melilotus alba* (16) and maize (35) differs somewhat from the foregoing cases, but a similarity exists with respect to the potential or demonstrated variation in chromosome number in the gametes of the parents.

As Müntzing (27) has pointed out, aneuploidy may be the basis for a "disturbed quantitative relation" between the chromosome numbers of embryo, endosperm, and surrounding maternal tissues. The normal quantitative relation between these tissues is 2n: 3n: 2n, and any disturbance in this normal relation is assumed to have severe effects on the development of seed. The frequent occurrence of shrivelled seeds in autotetraploid rye was explained on the basis of this "disturbed quantitative relation" (27), and this hypothesis is applicable to autotetraploid *Melilotus alba*.

Endosperm failure may be a primary cause of ovule abortion. This seems to be the case in autotetraploid *Secale* (14), in reciprocal crosses between 2n *Medicago falcata* and 4n *M. sativa* (21), and in the present subject, autotetraploid *Melilotus alba*. It is possible that the endosperm is particularly sensitive to the aneuploid condition.

Somatic tissues of the ovule are also involved in a type of ovule abortion. In self-pollinated *Medicago* the aggressive growth of the inner integument causes breakdown of the endosperm and subsequent abortion of the fertile ovule (3). This type of abnormality has been designated somatoplastic sterility. This phenomenon is of minor importance in autotetraploid *Melilotus alba*, in which very little excessive growth occurs in the inner integument or nucellus. Collapse of integumentary and nucellar tissues takes place in the ovules which collapse at advanced stages. The cells of the inner integument and nucellus in these collapsing ovules become more actively meristematic for a short time than in the normal

ovules. This excessive meristematic growth of nucellar tissue may be attributed to a shift of growth stimulus from the retarded endosperm to the surrounding maternal tissues.

Young (44), and Brink and Cooper (3) observed that after fertilization the "nutritive jacket," which is derived from the inner integument, becomes more strongly developed, especially in the chalazal region. In *Medicago,* collapse of fertile ovules takes place in proembryonic or early embryonic stages, whereas, in 4n *Melilotus alba,* well-developed embryos may collapse eight to ten days after pollination. In such collapsed ovules endosperm may be absent, or limited endosperm occurs in the free-nucleate condition.

In addition to the meiotic and post-zygotic abnormalities that are associated with sterility, pre-meiotic somatic aberrations deserve further study. The observed mitotic irregularities in cells of the young ovule can readily produce an aneuploid condition in the hypodermal initials from which the megasporocyte is derived. A study of the ovule primordium and perhaps the carpel primordium may reveal extensive somatic aberrations during the earlier phases of floral ontogeny.

The relationship between relative fertility and the observed mitotic and meiotic irregularities may serve as a guide in breeding programs designed to increase fertility in crop plants.

SUMMARY

A study of megasporogenesis, embryo sac development, and embryology of autotetraploid *Melilotus alba* was made to determine the cytological and the morphological basis of self-sterility. Two lines that had relatively high self-fertility and two lines of low self-fertility were used.

Irregularities at metaphase I consist of irregular orientation of bivalents on the metaphase plate, lagging of bivalents, and the frequent occurrence of univalents. These irregularities occur in high-fertility as well as low-fertility lines, but more frequently in low-fertility lines.

Three to four lagging chromosomes may occur at anaphase I in all lines studied. In low-fertility lines, the chromosomes of one polar group may be scattered irregularly in the surrounding cytoplasm, whereas the other polar group develops a typical interphase nucleus.

As many as six to seven lagging chromosomes may occur in telophase I, especially in the two low-fertility lines.

Lagging and non-orientation of chromosomes also occurs in metaphase and anaphase II. The chromosomes on one spindle may undergo normal separation, whereas separation on the other spindle lags or fails. The abnormal spindle may have a single heavily stained mass of chromatin, or its chromosomes may be in three to four compact, deeply stained groups. Anaphase separation on the two spindles of the diad is not always synchronous.

Irregular chromosome distribution also is evident in telophase II.

A total of 255 dividing cells were examined at anaphase and telophase

of I and II, and 183 cells (71.76 per cent) were found to have meiotic irregularities.

Dividing somatic cells of the ovule and ovary also exhibit lagging and exclusion of chromosomes. Abnormal somatic cells may well give rise to achesporial cells.

Collapse of unfertilized ovules may begin 36 hours after pollination and becomes marked by 48 hours.

Fertilization occurs under greenhouse conditions between 18 and 24 hours after pollination in both high- and low-fertility lines. Incidence of fertilization is higher in high lines than in low lines.

In the initial stages of embryonic growth, the highest-fertility line shows the highest rate of growth.

In normally developing ovules, the primary endosperm nucleus undergoes division 24 hours after pollination in high lines, and 36 to 48 hours after pollination in the lowest line. Normal endosperm becomes cellular six days after pollination.

Ovules with embryos in advanced stages of development ten days after pollination undergo collapse if the endosperm remains non-cellular.

Apparently normal ovules with proembryos may have an undivided primary endosperm nucleus six days after pollination. This condition is more pronounced in low lines.

Ovules which collapse at advanced stages, 8 to 10 days after pollination, have abnormal embryos.

Abnormal proliferation of integumentary tissue was observed in one high line and both low lines.

Collapse of fertilized ovules is consistently associated with failure or pronounced retardation of endosperm development.

Failure may take place at one of two critical ages, five to six days after pollination, or eight to ten days after pollination.

The foregoing irregularities of chromosome behavior, and abnormalities of embryo and endosperm development, provide the physical basis for the eventual collapse of fertile ovules and the subsequent failure of seed production.

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DEVELOPMENTAL MORPHOLOGY OF LOTUS CORNICULATUS L.[1]

Harold W. Hansen[2]

Departments of Botany and Agronomy
Iowa Agricultural Experiment Station
Iowa State College

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The introduction of new legumes and the improvement of established species have received considerable impetus in Iowa in recent years. In addition to the use of legumes as forage crops, their use in land renovation has been under investigation.

Lotus corniculatus L., birdsfoot trefoil, has been gaining popularity in certain parts of the United States because of its adaptability in regions that are not suitable for the growing of alfalfa, red clover, or white clover. Because of these advantages, trefoil has been incorporated in the legume breeding program in Iowa, for use in selected areas of the state.

A knowledge of the development history, anatomy, and cytology of a crop plant is of theoretical and practical value in a breeding program. The present study of *Lotus corniculatus* was undertaken to supply such basic information.

REVIEW OF PERTINENT LITERATURE

Among the dicotyledons, the *Leguminosae* rank second only to the *Compositae* in the number of species, and with regard to economic importance, the legumes are perhaps exceeded only by the *Gramineae*. A voluminous literature is available dealing with the taxonomy, distribution, and utilization of members of the *Leguminosae*.

Much of the early work on the morphology of the legumes dealt with the development of the floral structures. Payer (50) described the development of the flowers of *Trifolium ochroleucum, Lathyrus sylvestris,* and *Lupinus varius.* He found that a whorl of five sepal primordia arises first, followed by an alternating set of five petal primordia. The third whorl consists of stamen primordia which alternate with the petal primordia. The fourth whorl consists of stamens, on the same radii as the petals. The central portion of the floral axis develops a crescent-shaped carpel primordium which eventually closes and forms the pistil.

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Frank (23) described the development of the floral structures in *Medicago sativa, Trifolium pratense, Vicia cracca,* and *Lupinus elegans,* and stated that organ initiation is acropetal. Henslow (33) suggested that in taxonomic inquiries the developmental stages of the flower must be studied if the homologies of the fully developed structures are obscure. Henslow also described the origin and development of vascular strands within the floral organs. Schuepp (57) described floral development from the well-formed flower bud through the blooming phase in *Lathyrus,* but made no mention of the initiation of floral structures. Coe and Martin (12) described the mature floral organs of *Melilotus alba* in connection with their studies on seed production.

Over sixty years after the work of Payer (50), Grégoire (27) studied an unspecified *Lathyrus* and concluded that the carpel arises as a ring. Bugnon (8) studied organ initiation in *Lathyrus vernus, Trifolium pratense,* and *Lupinus perennis* and concluded that Grégoire had based his interpretation on an older stage of development in which the edges of the carpel had already fused. Guard (28) found the sequence and manner of origin of the floral structures in soybean to be essentially as described by Payer (50).

New impetus was given to the study of the flower by the formulation of the theory of carpel polymorphism by Saunders (55). Based on her study of carpel venation, Miss Saunders stated that the gynoecium in the legumes is usually composed of two carpels. The theory was vigorously opposed by Eames (21), Arber (2), Bugnon (9), and Moore (46), largely on the basis that Miss Saunders frequently used complex, specialized forms.

Hofmeister's (34) early investigations of a wide range of plant families included brief descriptions of the embryo sac, fertilization, and early embryo development of some legumes. Guignard (30) investigated many genera, including *Acacia, Mimosa, Cercis, Orobus, Pisum, Lathyrus, Lupinus,* and *Phaseolus.* He reported that in some instances the embryo sac develops directly from a subepidermal cell of the ovule primordium (*Medicago, Melilotus*); in other cases the subepidermal cell divides to form two cells (*Orobus, Pisum*), or three cells (*Acacia retinodes, A. decurrens, Gleditsia, Cassia, Faba, Genista, Cytisus,* and *Phaseolus*); in some species, a row of four cells is formed. He described the eight nucleate embryo sacs and the formation of the embryo and endosperm. Young (72) stated that, in *Melilotus alba,* the megasporocyte develops directly into the embryo sac without the formation of four megaspores. Coe and Martin (12) stated that, on the contrary, a megaspore quartet is formed in *M. alba,* and this was substantiated by Cooper (14). Martin (45) also showed that megaspores are formed in *Trifolium pratense, T. repens, T. hybridum, Medicago sativa,* and *Vicia americana,* and Cooper (15) and Reeves (52) demonstrated this process in *Medicago,* and Hanson (31) in *Lespedeza stipulacea.* Brown (7) and Weinstein (67) found that, in *Phaseolus vulgaris,* three megaspores are formed as a result of the failure of the division of one diad.

Guignard (29) described the embryology of a number of legumes. Based on his observations and on the accounts of others, he presented a system of classification of embryos. Souèges (60, 61) likewise investigated embryo formation in legumes and contributed to the clarification of embryo types.

The presence of so-called "hard seeds" among legumes has prompted investigations of the seed coat. Harz (32) and Pammel (49) described seeds and seed coats of a number of species. Reeve (51) made chemical analyses as well as microscopic studies of legume seeds.

The vegetative morphology of legumes has not received very extensive consideration. Janczewski (37) investigated root apices and placed *Pisum sativum* and *Phaseolus vulgaris* in a group that is characterized by a transverse generative layer which gives rise to the primary tissue systems. The stem apices of *Anthyllis* and *Lupinus* were studied by Douliot (19), who found that the central cylinder and the cortex arise from a common initial and that a second initial gives rise to the epidermis. However, in *Trifolium* and *Onobrychis* he found three distinct histogens, which give rise respectively to the epidermis, the cortex, and the central cylinder. In all four genera the leaf was found to arise from the two outer histogens. Wilson (69) described the structure of the fully developed stem and leaf of *Medicago sativa* and summarized in tabular form the size relationships of the various kinds of cells.

The histological structure of alfalfa, red clover, and alsike clover was compared by Winton (71). Her investigation was designed to facilitate the microscopic identification of these three species in mixed cattle feed. Winter (70) described the vascular system of young plants of *Medicago sativa* and Doutt (20) studied vascularization in *Phaseolus vulgaris*. The ontogeny of the primary axis of *Soja max* was described by Bell (3). He found that the root tip of this plant belongs to the group in which Janczewski (37) had placed *Pisum sativum* and *Phaseolus vulgaris*. Simonds (59) described the histological structure of the seedling and mature plant of alfalfa, with special emphasis on the crown.

Early investigations in seedling structure in the family were summarized by Compton (13), supplemented by original work. He emphasized the value of size, form, habit, and general morphology of a species with respect to phylogeny.

The nomenclature of *Lotus corniculatus* has an involved history. According to Brand (5), the name *Lotus* has been applied to several different entities, for instance, the water lily of the Nile, *Nymphaea lotus*, the lotus of the Homeric lotus-eaters, probably *Rhamnus lotus*, and the nettle tree *Celtis Australis*. Another lotus of the Greek meadows, probably a *Trifolium*, was used as fodder. *Lotus corniculatus* was described under the name *Lagopus primus* by Bock (4). The generic name *Lotus* was first used by Camerarius (10) and *Lotus incana* was described and pictured by Gerarde (25). Linne's (40) first edition of "Species Plantarum" included *Lotus corniculata* which subsequently appeared as *Lotus corniculatus* in the second edition (41). Gams (24) described the

genus as consisting of about 100 species in the Mediterranean area, some of which also occur in Middle and Northern Europe, Africa, Australia, and the Canary Islands.

According to Robinson (53), *Lotus corniculatus* is indigenous to the British Isles. MacDonald (43) reported that the species is not a native of the Western Hemisphere, but was introduced from Europe. The species has become naturalized in the United States, chiefly in New York, Oregon, and California. In New York, distribution apparently began from ballast dumps and impurities in imported seeds. In Oregon and California, imported seed is thought to have been the source.

Within the species *L. corniculatus,* two distinct types occur, a broad-leaved type and a narrow-leaved form designated *L. corniculatus* var. *tenuifolius* by some authors, and as a species, *L. tenuis,* by others.

Müller (47) described the mechanism of pollination in *Lotus corniculatus.* He found that pollen is shed in the base of the cone-shaped keel (carina) before the flower has reached full size. Delpino (18) was of the opinion that the stigma does not become capable of fertilization until its papillae have been slightly rubbed, a process which makes the surface sticky. Elliott (22) demonstrated the presence of a membrane covering these papillae in *Lotus tenuis.* Müller (47) and Knuth (39) indicated that a wide variety of insects visit the flowers, but MacDonald (43) stated that bees appear to be the only insects that operate the pollinating mechanism.

Souèges (62) described the early embryology of *Lotus corniculatus,* with particular emphasis on the proembryo. Johansen's (38) classification of embryo types places *Lotus* in the "Onagrad" group, which also includes *Trifolium.* The essential features of the group are: the zygote divides by a transverse wall; the terminal cell divides by a longitudinal wall; the basal cell has no part, or only a minor role, in the construction of the embryo proper.

Markova (44) presented in tabular form the relations between the dimensions of the floret buds and the stages of development of the female gametophyte, with a view to their use in determining dates suitable for hybridizing various legumes, including *Lotus corniculatus.*

Harz (32) and Nadelmann (48) described the pod and seed of *L. corniculatus,* and Schmidt (56) summarized data on sixteen species, recording information on seed size and shapes, dimensions of cell layers, and the composition of the endosperm and cotyledons.

The transition zone of birdsfoot trefoil was described by Chauveaud (11) and Compton (13). Early references, as well as the more recent publications of Goebel (26), Britton (6), Watari (66), and McKee and Schoth (42), stated that the leaf of *Lotus corniculatus* is trifoliate, and has a pair of basal stipules. Irmisch (35, 36), however, maintained that the basal "stipules" are in fact leaflets, and that two small brown spots at the base of the petiole are the stipules. On the young leaves these spots appear glandular, later they become somewhat acuminate, and finally blunt. Alefeld (1) maintained that the disputed structures are

true stipules, and considered the glandular spots as belonging to the stipules, though spatially detached. Vuillemin (65) described the tissue organization of the leaf of *Lotus corniculatus* and Schmidt (56) examined the leaves of a number of species of *Lotus*. The histological features are essentially as in many other herbaceous, mesomorphic legumes.

Fertility relationships in *L. corniculatus* have been studied extensively. Darwin (16) reported that the species produces no seeds when isolated from insects. Knuth (39) recognized that the species is largely self-sterile. Silow (58) stated that some plants are self-fertile, but that ten times as many seeds are formed when the plants are cross-pollinated. Tome and Johnson (63) also found the species to be highly self-sterile. MacDonald (43) concluded that *L. corniculatus* var. *vulgaris* is fully self-fertile when insect pollinated. Self-fertility appears to be variable, depending on the strain of plant and perhaps on the locality where grown. Contradictions in the literature may also be due in part to the lack of agreement in the identification of varieties and possible intermediate forms.

Tschechow and Kartaschowa (64) described and illustrated the chromosomes of species of *Lotus*. *L. corniculatus* was found to have a somatic number of twenty-four. *L. tenuis* Wald. and Kit. has the somatic chromosome number of twelve. Dawson (17) observed tetrasomic segregation in the inheritance of cyanogenetic properties in *L. corniculatus*. Bivalent associations were present at meiosis, and quadrivalents appeared to be rare. Dawson concluded that *L. corniculatus* had probably arisen as an autotetraploid from *L. tenuis* or a prototype. Tome and Johnson (63) succeeded in doubling the chromosome number in *L. tenuis* by the use of colchicine. They found, however, that the resulting autotetraploid did not resemble *L. corniculatus* with regard to the shape of the basal leaflets. Attempts at crossing the induced tetraploid with *L. corniculatus* did not yield viable seed. They concluded that the failure to obtain seed does not exclude the possibility that *L. corniculatus* arose as an autotetraploid of *L. tenuis*. Chromosome differentiation during a long period after the natural formation of the autotetraploid may have sufficiently separated this species from an experimentally produced autotetraploid to render them cross sterile.

MATERIALS AND METHODS

Plants of *Lotus corniculatus* var. *vulgaris* were used in this study. Field-grown materials were collected from well estabilshed plots at the Soil Conservation Service Nursery, Ames, Iowa, and from a small plot of the Botany Department, Iowa State College, Ames, Iowa. Type specimens are on file in the herbarium of Iowa State College.

Cuttings from field-grown plants were rooted in sand in the greenhouse and later transplanted to four-inch pots of soil. The plants were handled as described by Elliott (22). At three-week intervals, each pot was given 20 cc. of a nutrient solution of 5 g. KH_2PO_4, 5 g. KNO_3, and 2 g. $MgSO_4$ dissolved in a gallon of tap water. The stem tips of the

plants were cut back occasionally to promote branching of the plants.

On January 5, a twenty-hour photoperiod was initiated, using 200-watt clear Mazda lamps. On February 3, the photoperiod was extended to twenty-four hours. The first flowers opened on February 19, and the plants continued to blossom for approximately eight weeks.

For the studies of embryo development, clone #39 was used as the female parent and clone #3 as the male parent. (These numbers refer to clonal numbering in plots growing at the Soil Conservation Nursery, Ames, Iowa). Clone #39 was selected from an introduction from the Soil Conservation Nursery, Big Flats, New York. Clone #3 was selected from a strain obtained from Elsberry, Missouri, where the strain was designated as No. H 1-2642.

Pollinations were made at 2 P.M. during the period from February 19 to April 22. Freshly opened flowers were prepared for pollination by removing the distal portions of all the petals with forceps. The stigmatic surface was scarified by gentle stroking with a toothpick. The floret selected to furnish the pollen was removed from the plant, and the standard and wing petals were picked off. The remaining portions were placed on a clean microscope slide, and the keel was compressed in such a way that the pollen was forced out on the slide. The previously scarified stigma of the female plant was then drawn through this mass of pollen.

Collections of ovaries were made beginning twenty-four hours after pollination. In the early stages, the entire ovary was preserved. As the ovaries became larger and tougher, it was found necessary to subdivide them before processing. Extracted embryos were processed for the study of some of the advanced stages.

The viability of samples of pollen was determined by germination on an artificial medium which consisted of 0.5 g. agar and 1.0 g. sucrose in 25 cc. of tap water. The solution was boiled, poured into petri plates, and allowed to cool. Pollen was dusted on the surface and the plates were incubated at room temperature. Germination of the pollen could be observed within an hour.

The Nawaschin type formula (Craf III) (54) was found to be most useful for killing vegetative portions of the plant. Material used for the study of pollen formation, embryo sac and embryo development was immersed in Farmer's solution (3 vol. anhydrous ethyl alcohol and 1 vol. glacial acetic acid) until bleached white, and then transferred to the Nawaschin solution. All materials were fixed a minimum of five days, dehydrated in a dioxan-normal butyl alcohol series (54), embedded in paraffin, and sectioned. Woody stems and roots were soaked in warm water prior to cutting. Iron hematoxylin was used to stain materials where cellular detail was important. For general histological preparations, the best results were obtained with a tannic acid, hemalum, safranin, and fast green combination (54).

To insure more uniform germination, seeds were scarified by scratching with a needle. Seedling studies were made on materials germinated on moist filter paper in petri dishes kept in a 20°C. incubator.

Older seedlings were grown in sphagnum moss, sand, or soil in the greenhouse.

EXPERIMENTAL RESULTS

DESCRIPTION OF THE PLANT

Lotus corniculatus var. *vulgaris* Koch (Fig. 1) is a perennial plant with ascending or decumbent glabrous stems which may reach a length of two or more feet. Although the plants used in this investigation were relatively smooth, considerable hairiness is present in some strains.

The leaves are alternate. The first two leaves of the seedling plant are trifoliate, whereas the other leaves normally bear five leaflets (Fig. 2).

The root system of birdsfoot trefoil consists of a large taproot with numerous branches. Nodules are usually abundant on the roots of field-grown plants.

The inflorescence consists of a group of pediceled flowers borne at the apex of a long peduncle (Fig. 2). One hundred flowering stalks were examined, and the number of flowers was found to vary from one to seven, the average being five. The typically papilionaceous, diadelphous flowers are yellow and frequently have a reddish tinge. The ovary is a cylindrical tube consisting of a single carpel; the style is attached to the ovary at an angle of about 45 degrees. The stigma is a flattened terminal knob.

The fruit is a typical legume; the appearance of the spreading cluster of pods at the end of a long peduncle has given rise to the common name, birdsfoot trefoil (Fig. 2). When ripe, the pod splits along both sutures, and the two valves spring apart and become twisted spirally (Fig. 2). One hundred pods were examined and were found to contain from two to 35 seeds; the average number was 19.

The seeds of the plant are among the smallest of all the cultivated legumes. They are round or somewhat flattened and are about 1.5 mm. in length. Their color ranges from buff to olive brown.

INITIATION AND DEVELOPMENT OF THE FLORAL ORGANS

The transition from a vegetative to a flowering apex is initiated by a broadening and lobing of the apical meristem (Figs. 3, 4). The leaf which subtends this lobed apex remains trifoliate, in contrast to the usual leaf, which has five leaflets. At this stage of transition, each lobe on the apical meristem is the primordium of a flower. Near the base of each lobe, on the abaxial side, a ridge develops (Figs. 5, 9). This ridge, which may be regarded as a bract, arises in a manner similar to that of a leaf primordium, but the bract remains undeveloped.

The first floral whorl consists of five sepals which originate as small, separate elevations at the periphery of the flat-topped lobes (Fig. 6). The bases of the sepal primordia broaden until they meet, and the entire whorl grows up as the tubular portion of the calyx. The next whorl is composed of five petals which originate as small papillae alternate with the sepals (Fig. 7). The third whorl consists of five stamen primordia which arise

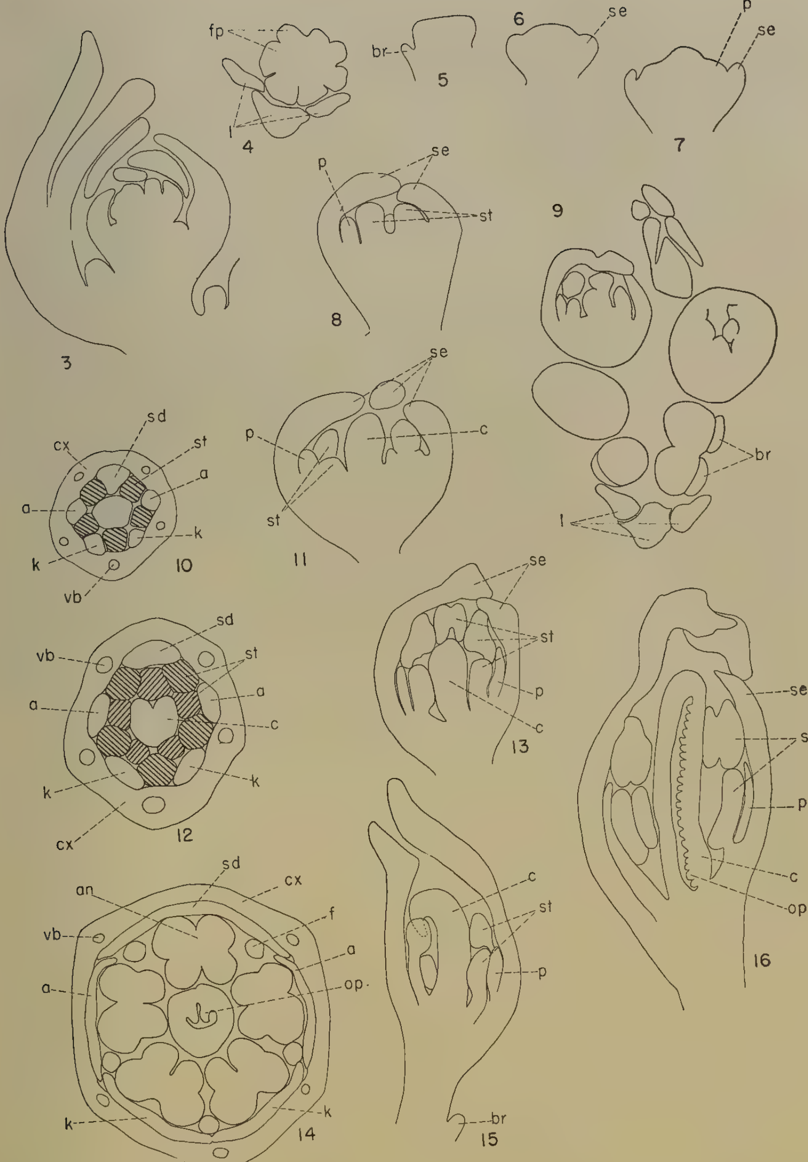
Fig. 1. Birdsfoot trefoil (*Lotus corniculatus* var. *vulgaris* Koch).

FIG. 2. Top row, left to right: leaves, inflorescence, single floret. Lower row, left to right: cluster of fruits, mature fruits, dehisced fruits with twisted valves.

FIG.

3. Longitudinal section of floral bud ($\times 40$).
4. Transverse section of floral apex ($\times 40$).
5. Longitudinal section of individual floral apex ($\times 80$).
6. Longitudinal section of floral apex showing sepal primordia ($\times 80$).
7. Longitudinal section of apex showing primordia of sepals and petals ($\times 80$).
8. Longitudinal section of flower bud showing sepal, petal, and stamen primordia ($\times 80$).
9. Oblique section through flowering head showing orientation of bracts ($\times 40$).
10. Transverse section of flower bud ($\times 80$).
11. Longitudinal section of flower bud showing primordia of members of five floral whorls ($\times 80$).
12. Transverse section of flower bud showing five floral whorls ($\times 80$).
13. Longitudinal section of flower bud showing lobing of anthers ($\times 40$).
14. Transverse section of flower bud through level of lower tier of anthers ($\times 40$).
15. Longitudinal section of flower bud showing adaxial bending of style ($\times 40$).
16. Longitudinal section of flower bud ($\times 40$).

a	ala
an	anther
br	bract
c	carpel
cx	calyx
f	filament
fp	flower primordia
k	keel
l	trifoliate leaf
op	ovule primordium
p	petal
sd	standard
se	sepal
st	stamen
vb	vascular bundle



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almost simultaneously with the petal primordia, but alternate with them (Figs. 8, 10). The members of the fourth floral whorl alternate with the members of the preceding whorl and make up the second whorl of stamen primordia (Fig. 11). The central portion of the floral apex continues to elongate, but at this stage remains morphologically undifferentiated. After the initiation of the floral whorls, localized meristematic activity transforms the cylindrical apical portion into a thick U-shaped carpel primordium (Fig. 12). At this stage, the rudimentary petals have broadened slightly into small arcs, as seen in cross section, but they remain shorter than the outer stamen primordia. The largest petal, the standard (banner or vexillum) is located opposite the two free edges of the carpel; there are two lateral wing petals (alae); the two remaining petals fuse along their abaxial edges to form the keel. Subsequent coalescence of the distal adaxial edges of the keel petals forms a tube around the anthers and style. A small pore remains at the apex of the tubular keel.

The stamens increase in length, become capitate and finally lobed (Fig. 13). The carpel likewise increases in length and becomes recurved adaxially (Fig. 15). Although the entire bud is less than two millimeters in length at this stage, the floral organs are well differentiated.

Growth of the region basal to the stamens brings about an apparent merging of the proximal ends of nine of the filaments. The tenth filament, which is a member of the original fourth floral whorl, is located between the free edge of the carpel and the standard primordium, and remains attached separately to the receptacle. The typical diadelphous arrangement of the stamens is thus brought about. The anthers are closely packed in two tiers, the distal one consisting of members of the third floral whorl and the more proximal one of members of the fourth floral whorl (Figs. 14, 16).

The floral organs continue to increase in size, and shortly after the petals extend beyond the tips of the sepals, the united base of the filaments elongates greatly and surrounds the base of the ovary as a collar, and the nine anthers are in turn elevated so that they surround the style. The tenth filament elongates simultaneously and thus maintains a development parallel to that of the other members of the whorl. The style, which also increases in length, extends to the tip of the keel and terminates in a slightly expanded stigma.

Development of the stamens and pollen. A stamen originates as a finger-like process (Fig. 17) which soon becomes capitate (Fig. 18), and the rudimentary anther becomes delimited from the filament. The outer layer of anther cells maintains its identity by dividing anticlinally. There is no apparent specialization of the cells in the central portion of the anther (Fig. 19) where the cells continue to divide in random planes. Eventually the anther becomes four-lobed (Figs. 20, 21, 22), and each lobe consists of a central region of sporogenous cells bounded by a tapetal layer. Between the tapetum and the epidermis are two rows of parietal cells (Fig. 23). Cell division in the sporogenous region continues to take place, and some increase in cell size occurs (Figs. 24, 25). The tapetal layer increases greatly in size and the cells adjacent to the

tapetum enlarge slightly, whereas the external parietal layer increases in size considerably. The epidermis remains relatively unchanged.

The random divisions of the sporogenous cells cease, and the definitive pollen mother cells enter the prophase of their first division (Fig. 26). The tapetum continues to enlarge, and the inner parietal layer becomes disorganized (Fig. 27). The microsporocytes complete the first and second division, the cells of the tapetum diminish in size, but wall formation between the microspore nuclei is delayed (Fig. 28). Eventually, wall formation takes place and delimits the four microspores (Fig. 29). The microspores increase in size and assume an oval shape (Fig. 30). They appear trilobed in end view due to the presence of three surface grooves. Remnants of the tapetal layer persist, and a striking development of the outer parietal cells takes place. These cells elongate radially and develop conspicuous V-shaped bars. The septum between the two adjacent pollen sacs disintegrates and the mature anther contains two large pollen cavities (Fig. 31). The tapetal layer disappears completely, leaving the mature, bi-nucleate pollen grains free, bounded by the specialized parietal cells (Fig. 32). Dehiscence occurs by means of longitudinal fissures.

Development of the carpel and ovules. The carpel primordium arises from the central dome-shaped mass of meristematic tissue (Figs. 10, 33, 34). The first differentiation of this floral organ occurs just after the four outer floral whorls have been initiated. A proliferation of the cells of the adaxial side brings about the formation of a U-shaped carpel primordium (Figs. 11, 12, 35). The rudimentary organ increases greatly in length, and at the time of lobing of the stamens, the terminal portion of the carpel is strongly recurved (Figs. 15, 36).

The carpel continues to grow, and the structure becomes roughly circular in section, with free edges (Fig. 37). Localized meristematic activity brings about the formation of two rows of minute papillae, the first indications of the developing ovules (Figs. 38, 40). The members of these rows are not directly opposite one another, but they are so numerous and crowded together that their exact arrangement is somewhat obscure. In later stages, their alternate arrangement may be recognized more easily.

The free edges of the carpel fuse at approximately the time that the pollen mother cells are well defined. The ovule primordia increase in length and extend into the cavity of the ovary (Figs. 39, 41). Further increase in ovule size is accompanied by bending, which brings the tip of the primordium to a position at right angles to the funiculus (Figs. 42, 43); the bending continues, resulting in the anatropous ovule (Figs. 44, 45, 51).

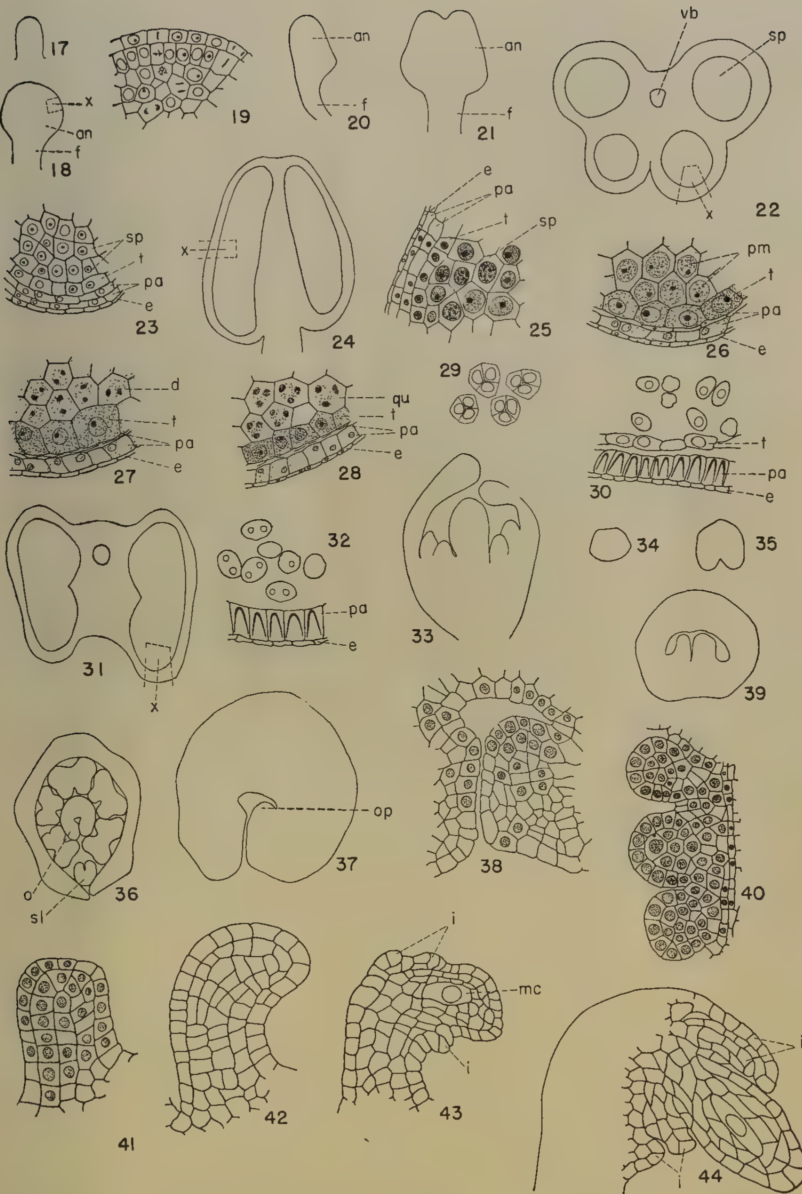
The megasporocyte arises as a hypodermal cell near the tip of the young ovule primordium (Fig. 43). The primordia of the two integuments become apparent soon after the sporocyte becomes distinguishable (Fig. 43). The integuments elongate (Figs. 44, 45) until they enclose the nucellus; a very narrow micropyle is present.

The megasporocyte undergoes two divisions, giving rise to a linear

FIG.

17. Longitudinal section of stamen primordium (×80).
18. Longitudinal section of stamen showing differentiation of anther and filament (×80).
19. Longitudinal section of anther; details of area "x" of Fig. 18 (×320).
20. Longitudinal section of stamen showing lobing of anther (×80).
21. Longitudinal section of stamen cut at right angles to section shown in Fig. 20 (×80).
22. Transverse section of young anther (×160).
23. Transverse section of young anther; details of area "x" of Fig. 22 (×320).
24. Longitudinal section of anther (×80).
25. Longitudinal section of anther; details of sector "x" of Fig. 24 (×320).
26. Portion of transverse section of anther showing pollen mother cells (microsporocytes) (×320).
27. Portion of transverse section of anther showing diads (×320).
28. Portion of transverse section of anther showing microspore quartets (×320).
29. Group of microspore quartets after formation of cell walls (×320).
30. Portion of transverse section of anther showing immature pollen (microspores) (×320).
31. Transverse section of mature anther (×80).
32. Transverse section of mature anther; details of area "x" of Fig. 31 (×320).
33. Longitudinal section of flower bud showing carpel primordium (×80).
34. Transverse section of carpel primordium (×80).
35. Transverse section of U-shaped carpel (×80).
36. Transverse section of flower bud showing tip of recurved style (×40).
37. Transverse section of open carpel (×160).
38. Transverse section of open carpel; details of portion of Fig. 37 (×320).
39. Transverse section of closed carpel showing ovule primordia (×80).
40. Portion of longitudinal section of carpel showing ovule primordia (×320).
41. Detail of elongated ovule primordium (×320).
42. Longitudinal sectional detail of curved ovule primordium (×320).
43. Longitudinal section of ovule showing megasporocyte and primordia of integuments (×320).
44. Longitudinal section of ovule primordium showing partially developed integuments (×320).

an anther
d diad
e epidermis
f filament
i integuments
mc megaspore mother cell
o ovary
op ovule primordium
pa parietal cells
pm pollen mother cells
qu quartet
sl style
sp sporogenous cells
t tapetum
vb vascular bundle
x sector shown in detail in next succeeding figure



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quartet of megaspores. The three micropylar cells disintegrate, leaving the chalazal cell as the functional megaspore (Fig. 46). The cells of the micropylar portion of the nucellus also disintegrate, and the area becomes occupied by the developing embryo sac. A series of nuclear divisions, beginning with the functional megaspore, brings about the formation of an eight-nucleate embryo sac (Figs. 47, 48, 49). One nucleus from each of the opposite ends of the embryo sac migrates to a central position. The mature female gametophyte consists of an egg and two synergids at the micropylar end, two polar nuclei in the central region, and three antipodal nuclei at the chalazal end (Figs. 50, 62). The chalazal end of the embryo sac extends into the adjacent maternal tissue, which makes identification of the nuclei in that region difficult. Fusion of the two polar nuclei takes place prior to fertilization. A slight trace of the nucellus remains in the form of a few deeply staining fragments (Figs. 50, 52). With the exception of this small area, the surface of the embryo sac is directly in contact with the innermost cells of the integument (Figs. 51, 52).

One hundred ovaries were removed from blossoms and were dissected. The number of ovules per ovary was found to range from 44 to 72, with an average of 59.

EMBRYOLOGY AND SEED DEVELOPMENT

In plants kept under greenhouse conditions at approximately 65°F., pollen tubes were found in the stylar canal 36 hours after pollination (Fig. 63). Pollen tubes were also found extending downward along the placental wall of the ovary, and in the micropyle. In material collected 48 hours after pollination under the above conditions, a well-defined zygote was present (Fig. 53), remnants of the pollen tube were evident, but the synergids and antipodals had disappeared (Fig. 64). The endosperm nucleus had already divided several times, and the nuclei were free in a peripheral mass of cytoplasm.

The zygote divides transversely and produces a two-celled proembryo approximately 60 hours after pollination (Figs. 54, 65). The basal cell divides transversely, whereas the distal one divides longitudinally (Fig. 55). Proembryos of this size were found three to five days after pollination. Successive division of the terminal cells produces a ball-shaped structure which eventually gives rise to the embryo proper; divisions of the two lower cells give rise to a stalklike suspensor and a basal cell (Figs. 56, 66). The dermatogen is derived by periclinal divisions of cells of the peripheral row near the apex (Figs. 57, 58). Later divisions in the outer row of cells occur only in an anticlinal direction, whereas divisions in the central portion of the globe continue to take place in random planes. A large number of free endosperm nuclei are present at five days after pollination.

Ten days after pollination a large globe-shaped proembryo is present and the endosperm has become cellular (Figs. 59, 60, 61). The endosperm does not fill the ovule completely, but surrounds a central vacuolar space in the same manner it did prior to wall formation. The outer row of

integumentary cells is palisade-like and is filled with deeply staining material; the cells of the second row enlarge considerably (Fig. 67).

The proembryo loses its radial symmetry about two weeks after pollination, when it broadens at the apex (Fig. 68) and later becomes heart-shaped (Fig. 69). The two lobes are the primordia of the cotyledons. A central stelar core or plerome becomes distinguishable in the hypocotyl.

Eighteen days after pollination the cotyledons are somewhat elongated (Fig. 70); the tips recurve and eventually become doubled back roughly parallel to the hypocotyl (Fig. 71). The region between the bases of the cotyledons shows no indications of the plumule at this time (Fig. 72). The cells in this region, however, are highly meristematic and exhibit the staining reactions of a meristematic stem apex. The tip of the hypocotyl now has the cellular organization of a root tip (Fig. 73).

Further development of the seed coat is seen in the modification of the second row of integumentary cells. These cells develop localized thickenings on their radial walls and begin to assume the "hour-glass" shape which is characteristic of this osteosclerid layer in legumes. The remaining layers of the integument decrease in size (Fig. 74).

Mitotic activity in the region between the bases of the two cotyledons brings about the formation of a dome-shaped apical meristem (Fig. 75). Twenty-four days after pollination, this dome develops a lateral bulge, which is the primordium of the first foliage leaf (Fig. 76). The entire embryo has increased in size, and differentiation has taken place in the stelar region (Figs. 77, 78, 79). A section cut 260 microns from the tip of the root shows three protophloem initials equally spaced in the stele. Indications of the triarch xylem arrangment are also present, but as yet the elements are not clearly differentiated. The cells of the cortical region appear in radial rows; a single hypodermal layer and a distinct epidermis are present. In a seed of this age, the walls of the osteosclerid layer have thickened, and a "light-line" has become apparent in the palisade layer of the seed coat (Fig. 80).

Between 24 and 30 days after pollination, the embryo increases in size, changes considerably in staining reaction, and the cells become filled with globular inclusions. No new features appear in the radicle or the seed coat.

DEVELOPMENT OF THE VEGETATIVE ORGANS

Development of the root. Scarified seeds germinate after three days in an incubator at 20°C. The emerged radicle has an open type of promeristem, with a common transverse meristematic zone which extends across the apex of the root (Figs. 81, 82). The cells at the apex of the central cylinder appear to be continuous with the longitudinal rows of root cap cells, and divisions of this zone produce the conical portion of the root cap as well as the stelar tissue. Divisions of the marginal portion of the transverse meristem contribute to the lateral regions of the cap and also bring about the formation of the dermatogen and periblem initials.

FIG.

45. Diagram of ovule showing completely developed integuments and micropyle (×160).
46. Functional megaspore and three disintegrating cells of the tetrad (×320).
47. Two-nucleate embryo sac developed from functional megaspore (×320).
48. Four-nucleate embryo sac (×320).
49. Eight-nucleate embryo sac (×320).
50. Mature embryo sac (×320).
51. Diagram of entire ovule showing mature embryo sac (×160).
52. Transverse section of mature ovule (×160).
53. Zygote with an adjacent endosperm nucleus (×320).
54. Two-celled proembryo, 60 hours after pollination (×320).
55. Four-celled proembryo, 72 hours after pollination (×320).
56. Proembryo, five days after pollination (×320).
57. Proembryo, eight days after pollination (×320).
58. Proembryo, nine days after pollination (×320).
59. Proembryo, ten days after pollination (×320).
60. Transverse section through ball-shaped portion of proembryo, ten days after pollination (×320).
61. Transverse section through stalk of embryo, ten days after pollination (×320).

at antipodals
eg egg nucleus
en endosperm
es embryo sac
ii inner integument
i integument
m micropyle
n nucellus
oi outer integument
po polar nuclei
sy synergids


ol
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45
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FIG.

62. Longitudinal section of ovule showing polar nuclei, egg, and one synergid in embryo sac (×400).
63. Longitudinal section of style showing pollen tube in stylar canal, 36 hours after pollination (×400).
64. Longitudinal section of ovule showing pollen tube near egg, 48 hours after pollination (×400).
65. Longitudinal section of ovule showing two-celled proembryo and endosperm nuclei, 60 hours after pollination (×400).
66. Longitudinal section of ovule showing small proembryo, five days after pollination (×400).

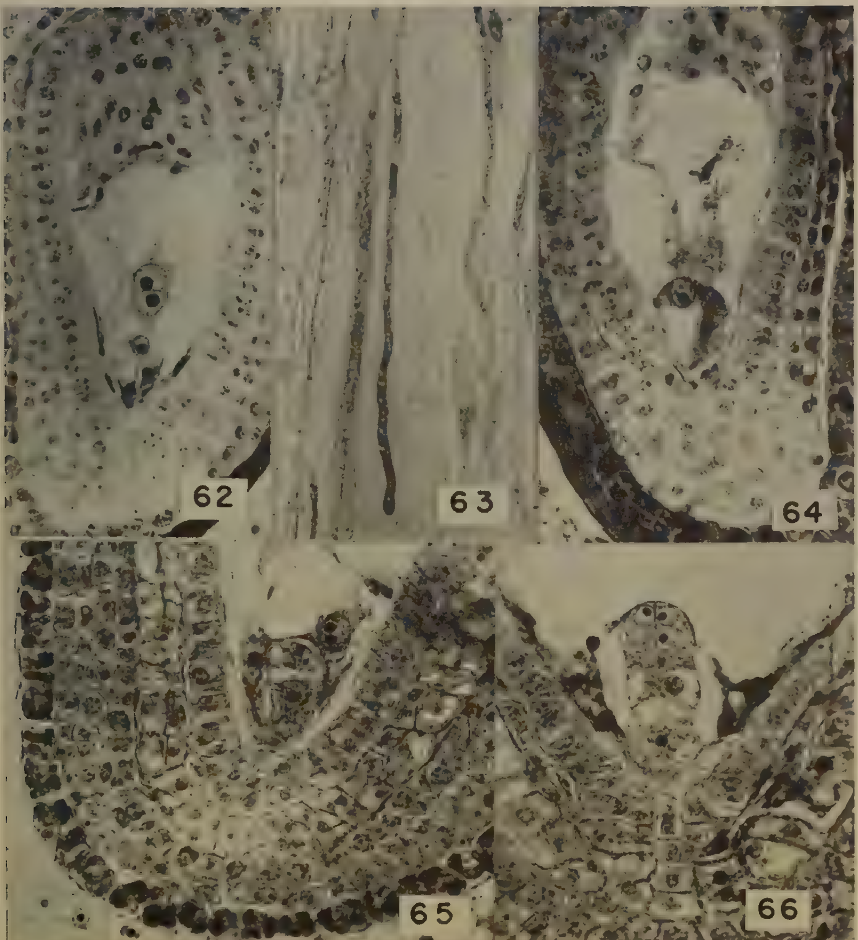
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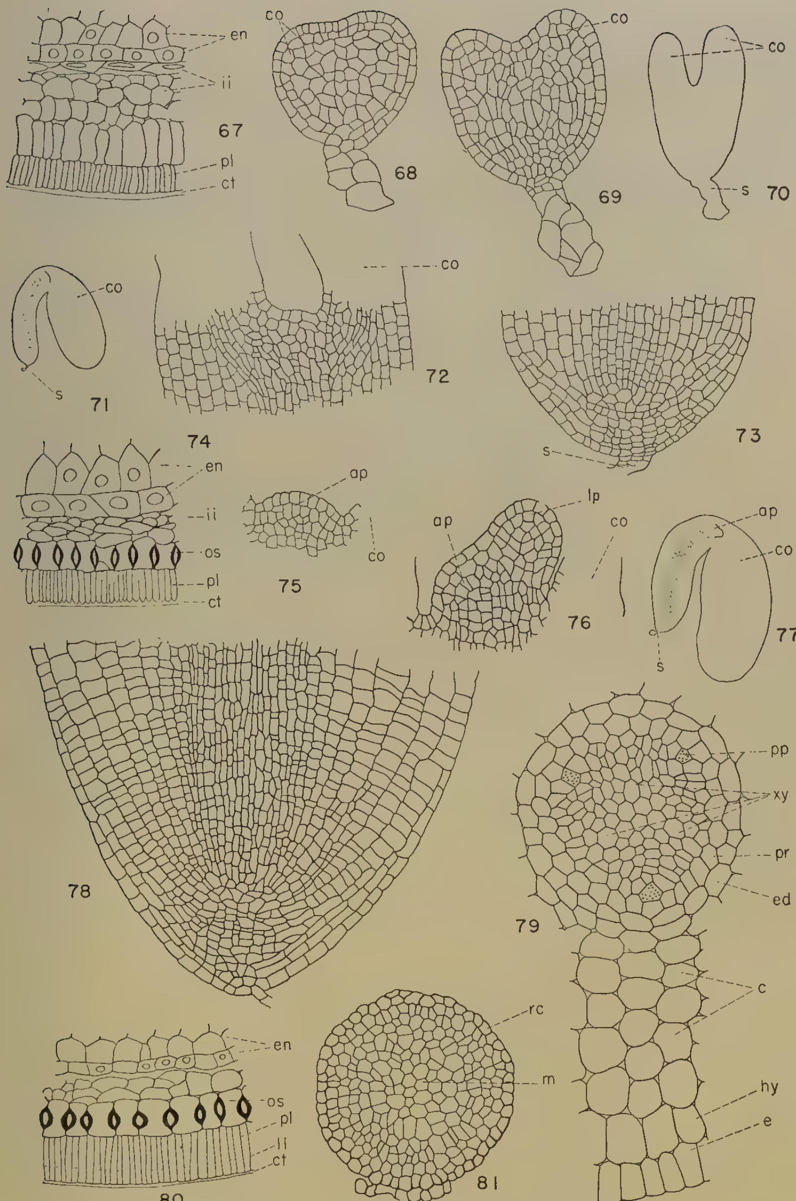
Fig.

67. Portion of cross section of seed coat, ten days after pollination (×160).
68. Embryo, 14 days after pollination (×160).
69. Embryo, 15 days after pollination (×160).
70. Embryo, 18 days after pollination (×80).
71. Embryo, 20 days after pollination (×16).
72. Detail of region at base of cotyledons, longitudinal section, 20 days after pollination (×160).
73. Detail of tip of hypocotyl, longitudinal section, 20 days after pollination (×160).
74. Portion of cross section of seed coat, 18 days after pollination (×160).
75. Longitudinal section through region at base of cotyledons, showing formation of stem apex, 22 days after pollination (×160).
76. Longitudinal section of stem apex of embryo, 24 days after pollination (×160).
77. Longitudinal section of entire embryo, 24 days after pollination (×16).
78. Longitudinal section of root tip of embryo, 24 days after pollination (×160).
79. Transverse section of root of embryo showing protophloem initials, 24 days after pollination (×240).
80. Portion of cross section of seed coat, 24 days after pollination (×160).
81. Transverse section of root apex, 24 days after pollination (×160).

ap apical meristem
c cortex
co cotyledon
ct cuticle
e epidermis
ed endodermis
en endosperm
hy hypodermis
ii inner integument
ll light line
lp leaf primordium
m meristem
os osteosclerid layer
pl palisade layer
pp protophloem
pr pericycle
rc root cap
s suspensor
xy immature xylem



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Just distal to the meristematic region, the cells of the calyptra are roughly isodiametric, whereas the lateral elements tend to be elongated in the direction of the root axis. The cells of the dermatogen elongate in a radial direction, and the identity of the layer is maintained by anticlinal divisions. After extensive elongation in the direction of the root axis, the dermatogen cells become the epidermis. The adjacent rows of cells making up the periblem are compact and devoid of intercellular spaces. After a limited amount of meristematic activity, they become differentiated into the cortex, a region characterized by large, thin-walled vacuolate cells with intercellular spaces. The innermost layer of cells derived from the periblem becomes the endodermis. The cortical region external to the endodermis (or to its precursor) has a definite cambiform stratification (Figs. 82, 85) brought about by predominantly tangential cell divisions.

A transverse section cut 390 microns from the root tip shows early differentiation of the stelar elements. Three protophloem initials, spaced at approximately 120 degrees from each other, are recognizable because of the plasmolyzing effect of the reagents on their specialized cytoplasm (Fig. 85). Three points of protoxylem are also visible in this section, although no lignification of the walls of the elements is evident. Small arcs of parenchymatous cells occur between the xylem and phloem initials. The outer layer of the stele consists of a single row of pericyclic cells.

In a cross section cut approximately one-half centimeter from the tip of an older root, thickening of the walls of the protoxylem elements is evident (Fig. 84). Differentiation of the primary xylem occurs centripetally and results in the formation of a solid core of xylem, characteristic of a typical radial protostele. In the metaphloem, sieve tubes, companion cells, and phloem parenchyma cells are present. The endodermis has Casparian strips. The cortical cells increase in length axially and lose their symmetrical appearance and arrangement as seen in cross section. Root hairs arise as extensions of epidermal cells.

Secondary growth in diameter of the root is apparent in sections cut three centimeters from the tip of the root of a 20-day-old seedling. The arc of parenchymatous cells between the pholem and xylem continues to divide tangentially, forming xylem elements centripetally and phloem elements centrifugally (Fig. 83). This activity extends laterally until a continuous cabium is formed. In an older root, the triarch configuration becomes obscured and a complete vascular cylinder of xylem and phloem is formed (Fig. 102). A parenchymatous ray extends outward from each of the original protoxylem areas; secondary rays also occur in the xylem and phloem. Coincident with the appearance of the cambium, small groups of fibers develop in the outer portion of the phloem (Fig. 101). The first indications of lateral root initiation can be recognized in the pericycle just external to the protoxylem points.

In older roots, periderm is initiated by tangential division in the pericycle. The phellogen produces layers of cork externally as well as

a limited amount of parenchymatous phelloderm internally. Cork formation brings about the rupturing, disintegration, and eventual sloughing of the epidermis and primary cortex. As the root increases in diameter through successive years, additional periderm layers are activated in the parenchymatous cells of the phloem; successive layers of the old phloem are thus sloughed. The xylem of a three-year-old root consists of pitted tracheal elements and parenchyma; the phloem includes sieve tubes, companion cells, "libriform" phloem fibers, and parenchyma. The vascular cylinder is surrounded by an irregular, narrow zone of phelloderm, phellogen, cork, and sloughed phloem elements.

Development of the stem. After 72 hours in a germinator at 20°C., resumption of growth of the embryonic leaf and stem apex in a seed is evident. Localized activity near the base of the leaf primordium brings about the formation of two lobes which become the lateral leaflets of the first trifoliate leaf (Fig. 86). A bud is initiated in the axil of each cotyledon by accelerated cell division (Fig. 89). Two histogens may be recognized at the stem apex, the tunica and the corpus. The tunica, which is continuous over the stem as well as over the axillary buds, consists of a single layer of cells which divide only anticlinally. Cell divisions in the corpus occur in random planes (Figs. 90, 97).

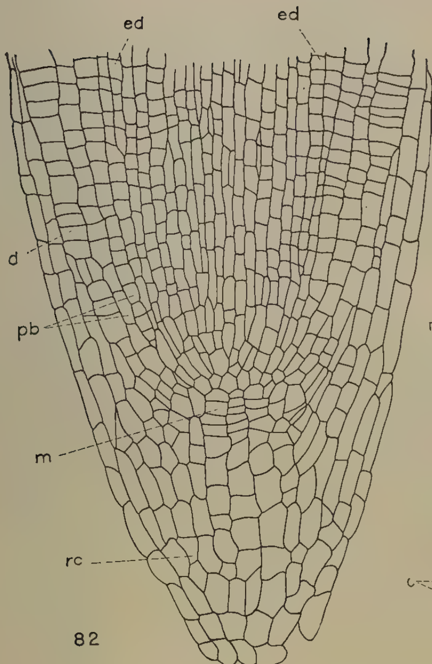
In a cross section cut 60 microns from the stem tip, four procambium strands are visible (Fig. 91); additional strands differentiate later. The cells of these strands undergo some axial elongation, but they remain relatively small in diameter; they contain dense cytoplasm, large nuclei, and they continue to divide actively. The cells of the surrounding ground meristem increase in size in all directions and become vacuolate.

A cross section of an older portion of the stem shows that the increase in size of the vascular strand takes place chiefly by division of the cells of the strand. Increase in the size of the surrounding tissues is due to cell enlargement. The contrast between the strand and the adjacent tissue thus becomes even more apparent. A cell near the outer margin of the strand becomes differentiated into a protophloem element; adjacent cells undergo similar specialization, forming a small region of phloem. In a slightly older portion of the stem, annular thickenings appear in the protoxylem elements near the inner margin of the strand (Fig. 87). The cells of the region between the xylem and phloem continue to divide; a predominance of tangential divisions gives the region a stratified appearance, and eventually gives rise to the fascicular cambium (Figs. 98, 99). Later, some of the outermost phloem elements elongate and develop thick walls, thus becoming phloem fibers. Occasional parenchymatous cells near the bundle are filled with deposits of a deeply staining material. External to the fibers, and forming the inner boundary of the cortex, is a single row of endodermal cells. The cells of the cortex are large, thin-walled, vacuolate, and have intercellular spaces. The outer one or two layers contain chloroplasts.

The cells of the primary rays undergo cambiform meristematic activity, and interfascicular cambium is thereby formed between the

FIG.
82. Longitudinal section of tip of radicle of seedling (×240).
83. Transverse section three centimeters from the tip of the radicle, 20-day-old seedling (×240).
84. Transverse section of young primary root approximately one-half centimeter from tip (×240).
85. Transverse section of primary root 390 microns from tip (×160).

c	cortex	p	phloem
ca	cambium	pb	periblem
d	dermatogen	pp	protophloem
e	epidermis	pr	pericycle
ed	endodermis	px	protoxylem
f	fibers	rc	root cap
m	promeristem	xy	immature xylem
mx	metaxylem		



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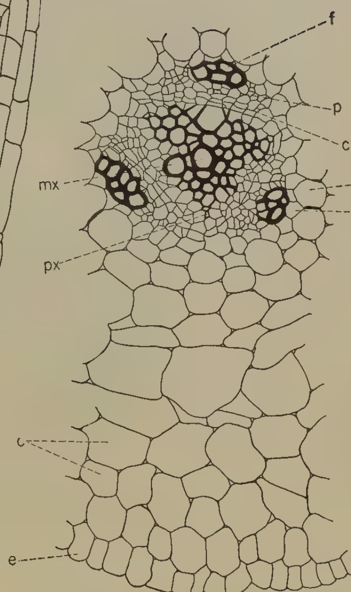

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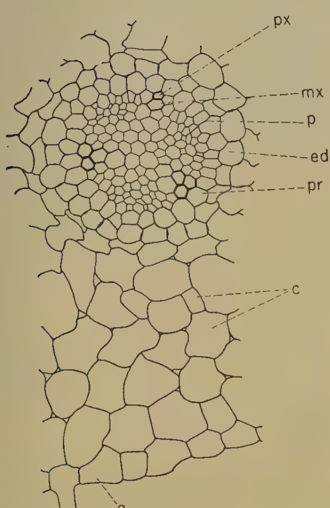

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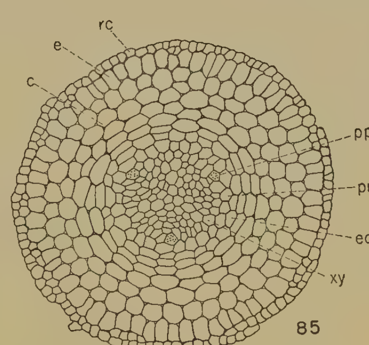

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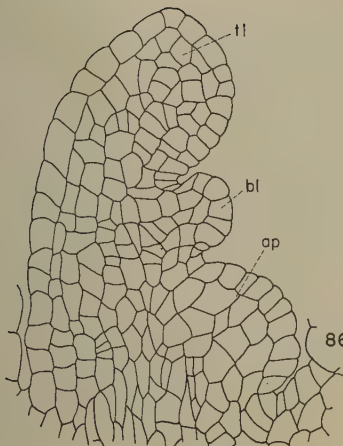
FIG.
86. Longitudinal section of stem apex between bases of cotyledons, 72 hours after placing seed in germinator (×320).
87. Transverse section of young stem showing primary phloem and primary xylem (×320).
88. Longitudinal section of stem apex showing primordia of five leaflets of one leaf (×240).
89. Longitudinal section of region at base of cotyledons, showing axillary buds, 72 hours after placing seed in germinator (×320).
90. Longitudinal section of stem apex of field-grown plant (×240).
91. Transverse section 60 microns from the tip of stem apex (×240).
92. Transverse section of leaves surrounding stem apex (×40).

ap apical meristem
ax axillary bud
bl lateral leaflet of trifoliate leaf
b1 basal leaflet, leaf no. 1
b2 basal leaflet, leaf no. 2
b3 basal leaflet, leaf no. 3
b4 basal leaflet, leaf no. 4
c cambium
co cotyledon
cs corpus
cx cortex
e epidermis
l1 lateral leaflet, leaf no. 1
l2 lateral leaflet, leaf no. 2
l3 lateral leaflet, leaf no. 3
l4 lateral leaflet, leaf no. 4
l5 lateral leaflet, leaf no. 5.
lp leaf primordium
m1 midrib, leaf no. 1
p phloem
pc procambium strand
pl palisade layer
px protoxylem
x sector shown in detail in Fig. 95.
t tunica
tl terminal leaflet
2t terminal leaflet, leaf no. 2



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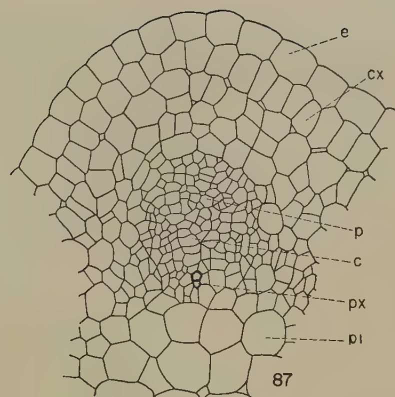

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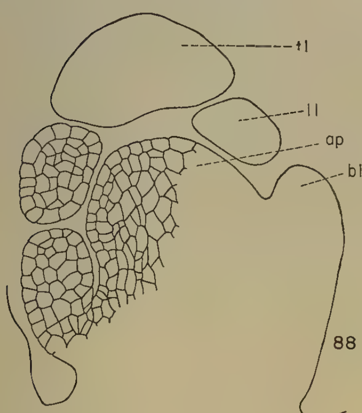

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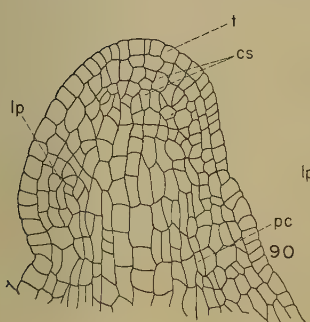

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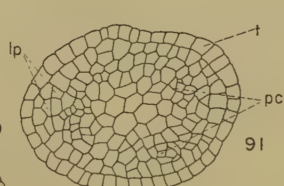

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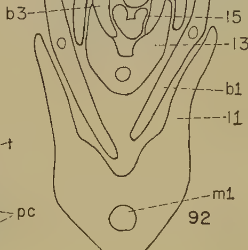

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bundles (Fig. 100). Centripetal differentiation of the cambial derivatives brings about the formation of a continuous band of secondary xylem which consists of pitted vessels, pitted tracheids, xylem rays, and an occasional vertical strand of parenchyma cells. The band of secondary phloem external to the cambium consists of sieve tubes, companion cells, and parenchyma.

The central region of the stem is occupied by thin-walled pith parenchyma cells. In the basal internodes of old plants, disintegration of these cells sometimes results in the formation of hollow stems. Phellogen arises

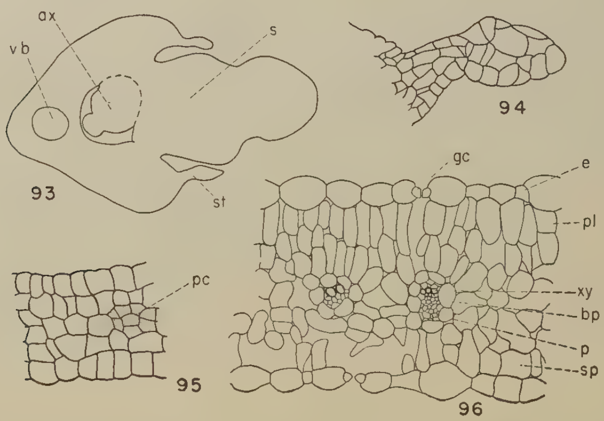

FIG.
93. Transverse section of stem at point of junction of petiole, showing stipules (×40).
94. Detail of stipule (×160).
95. Transverse section of portion of blade of young leaf (×320).
96. Transverse section of blade of mature leaf (×160).

ax axillary bud
bp border parenchyma
e epidermis
gc guard cell
p phloem
pc procambium strand
pl palisade layer
s stem
sp spongy parenchyma
st stipule
vb vascular bundle
xy xylem

in the cortex and produces cork externally and some parenchymatous phelloderm internally. In old stems, additional periderm develops in the older portion of the phloem. Breakage and disintegration occurs in the tissues external to the periderm.

Development of the leaf. The five-parted foliage leaf of *Lotus corniculatus* arises as a simple lobe near the stem apex. Initiation of the leaf takes place in the outer corpus, where accelerated mitotic activity gives rise to a crescent-shaped ridge. Anticlinal divisions occur in the cells of the tunica, thus maintaining the continuity of that layer in the leaf primordium and the stem apex (Figs. 90, 91). The developing leaf

extends upward and partly arches over the stem apex; the base of the petiole is broad and is adnate approximately half-way around the young stem.

Marginal activity in the leaf primordium brings about the formation of the lobes which become the leaflets (Fig. 88). The leaflets remain folded around the stem tip in such manner that the basal pair is innermost, followed by the lateral pair, and finally by the single terminal leaflet (Fig. 92).

The leaflet primordia broaden primarily by meristematic activity of the marginal meristem. At an early stage, each leaf primordium consists of five to seven layers of isodiametric cells, all rather similar in appearance, with the exception of small cells of the procambium strand (Fig. 95). Scattered among the polygonal epidermal cells are smaller, denser cells which appear triangular or rectangular in surface view. These smaller cells divide, each forming two adjacent guard cells. In the mature lamina, two layers of palisade parenchyma are present near the adaxial surface. The spongy mesophyll consists of approximately three layers of irregular parenchymatous cells. The smaller vascular bundles consist of a few xylem and phloem elements, surrounded by a layer of border parenchyma (Fig. 96). In the larger bundles, a cambiform layer is present between the xylem and the phloem. Two small processes, the stipules, which lack vascular connections, are present at the base of the petiole (Figs. 93, 94).

DISCUSSION

The sequence of development of the floral whorls of *Lotus corniculatus* was found to be similar to that of other legumes which had been investigated by Payer (50), Frank (23), Scheupp (57), Bugnon (8), and Guard (28). The whorls arise in acropetal succession.

The carpel was found to arise as an unclosed ring which eventually closes by the fusion of the adaxial margins. This coincides with the earlier investigations of members of the family, with the exception of the work of Grégoire (27) on *Lathyrus*. His interpretations were probably erroneous, for, as pointed out by Bugnon (8), Grégoire's observations did not extend back into the earliest ontogenetic stages of the flowers.

Henslow (33) stated that the stamens, though diadelphous in the fully developed flower of *Lotus corniculatus*, are monadelphous at first, and the tenth stamen subsequently becomes free by the atrophy of the tissue on either side of the filament. The present investigation shows that the ten stamens originate as separate primordia. At a later stage of development, growth of the region just basal to nine of the filaments brings about an apparent merging of these members, whereas, the process is actually an elevation of the stamens. The adaxial tenth filament remains attached separately to the receptacle.

The formation of four megaspores, of which the chalazal cell survives and gives rise to an eight-nucleate embryo sac, seems to be typical in the *Leguminosae*. This type has been observed in *Leguminosae* by

Fig.

97. Longitudinal section of terminal bud showing vegetative stem apex, leaf primordia and developing leaves (×62).
98. Transverse section of young stem with primary tissues (×62).
99. Details of sector from stem shown in Fig. 98 (×148).
100. Portion of transverse section of stem in which interfascicular cambium has become active (×121).
101. Sector of transverse section of old root, showing periderm (×148).
102. Transverse section of root, showing extensive secondary tissues and periderm (×62).


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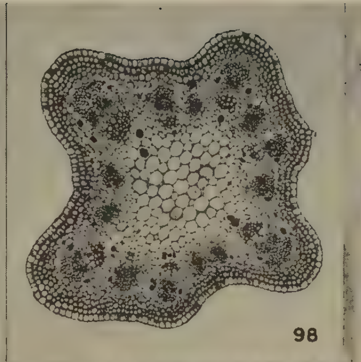
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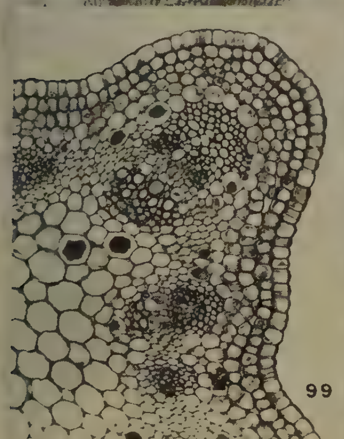
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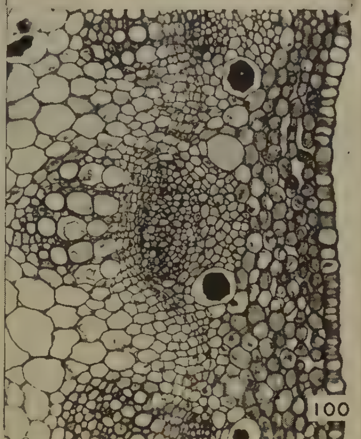
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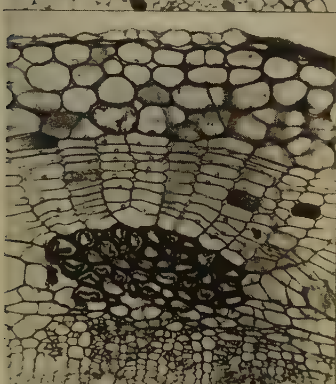
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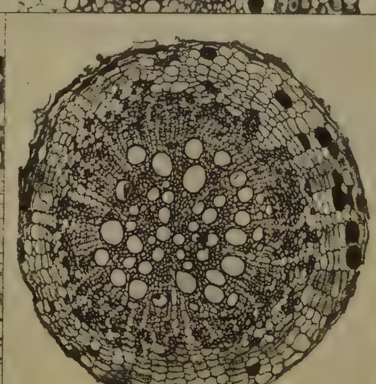
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Martin (45), Coe and Martin (12), Reeves (52), and Cooper (15), as well as in the present study.

The cellular organization of the root tip corresponds to the type described by Janczewski (37) as the predominant type in *Leguminosae*.

The rows of cortical cells in the root appear to arise from meristematic activity of the endodermal layer. This is in accord with the work of Williams (68), who found evidence of this type of growth in 195 species, including monocotyledons, dicotyledons, pteridophytes, and gymnosperms.

Investigators engaged in the study of fertility relationships in *Lotus* may find the method of germinating pollen on agar to be of value in determining pollen viability. A comparative study of the early embryology of various lines, varieties, and species of *Lotus* may be of assistance in understanding the failure of embryo development in certain crosses. As Johansen (38) has observed, the zygote of interspecific crosses may divide once, and then perish. The first division of the zygote is universally transverse and presents no difficulties, but variations occur in the plane of the succeeding division. If the hybrid embryo survives beyond the first division and then fails, it may be an indication that the two parents belonged to two different developmental types. Comparative investigations of the formation of the endosperm may also shed light on the causes of embryo abortion in interspecific crosses.

SUMMARY

A study was made of the developmental morphology of *Lotus corniculatus* var. *vulgaris* Koch.

The transition from a vegetative to a flowering apex is initiated by a broadening and lobing of the stem tip. Each lobe is a flower primordium, subtended by a small bract.

The five whorls of floral organs arise in acropetal succession. Each floral organ arises as a separate primordium. The calyx tube is formed by an annular meristem at the base of the ring of sepal primordia. The petal primordia produce free petals, except that two petals fuse and form the keel.

The stamens originate as slender processes which become capitate and finally four-lobed. Each lobe contains a central column of sporogenous cells, surrounded by a tapetum. A microsporocyte undergoes the two divisions of meiosis prior to the formation of cell walls. Each microspore becomes a binucleate pollen grain. Anther dehiscence occurs by means of longitudinal sutures.

The carpel arises as a dome of meristematic tissue, becomes U-shaped and eventually closes to form a one-carpellate ovary. A row of ovule primordia arises along each edge of the young carpel. The megasporocyte differentiates from a hypodermal cell.

The megasporocyte undergoes the two divisions of meiosis and produces a linear quartet of megaspores. The chalazal megaspore gives rise to the female gametophyte, which consists of an egg, two synergids, two

polar nuclei, and three antipodals. The nucellus disappears and fusion of the polar nuclei occurs prior to fertilization.

In cross-pollinated flowers, under greenhouse conditions, pollen tubes were demonstrated in the stylar canal and micropyle 36 hours after pollination. Forty-eight hours after pollination, a well-defined zygote and free-nuclear endosperm were present.

The early proembryo consists of a linear row of cells; accelerated cell division at the distal end produces a ball, which later becomes bilobed. Each lobe is the primordium of a cotyledon. Differentiation of histogens of the hypocotyl precedes the formation of the embryonic plumule. The axis of the mature embryo has a stem apex, the primordium of the first leaf, and the radicle, which has three protophloem initials and indications of the triarch protoxylem.

The seed coat consists of a palisade layer and the osteosclerid layer, which develop from the outer two rows of integumentary cells. The inner layers of the integuments remain parenchymatous. A layer of endosperm is present.

The root of the seedling has an open type of promeristem, with a transverse meristematic zone. The primary vascular system is a typical radial protostele. Phellogen layers develop first in the pericycle and later in phloem.

Two continuous histogens, the tunica and the corpus, are present in the stem apex, the leaf primordia, and the axillary buds. Procambial strands differentiate near the stem apex; protophloem elements are visible prior to the thickening of the walls of the photoxylem elements. Fascicular cambium becomes active in very small stems. Interfascicular cambium arises from primary rays. Cambial activity produces a continuous vascular cylinder of xylem and phloem. Periderm develops in the inner layers of cortex of old stems.

A foliage leaf arises as a simple lobe, which becomes five-parted by differential activity of the marginal meristem. At an early stage, the blade consists of five to seven layers of relatively undifferentiated cells. At maturity, two layers of palisade parenchyma and approximately three layers of spongy cells are present. Stomates are present on both upper and lower surfaces.

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